U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-253 528

Toxicity to Fish of Cyanides and Related Compounds: A Review

Oregon State Univ.

Prepared For
Environmental Res. Lab.

April 1976

KEEP UP TO DATE

Between the time you ordered this report—which is only one of the hundreds of thousands in the NTIS information collection available to you—and the time you are reading this message, several *new* reports relevant to your interests probably have entered the collection.

Subscribe to the **Weekly Government Abstracts** series that will bring you summaries of new reports *as soon as they are received by NTIS* from the originators of the research. The WGA's are an NTIS weekly newsletter service covering the most recent research findings in 25 areas of industrial, technological, and sociological interest—invaluable information for executives and professionals who must keep up to date.

The executive and professional information service provided by NTIS in the **Weekly Government Abstracts** newsletters will give you thorough and comprehensive coverage of government-conducted or sponsored research activities. And you'll get this important information within two weeks of the time it's released by originating agencies.

WGA newsletters are computer produced and electronically photocomposed to slash the time gap between the release of a report and its availability. You can learn about technical innovations immediately—and use them in the most meaningful and productive ways possible for your organization. Please request NTIS-PR-205/PCW for more information.

The weekly newsletter series will keep you current. But *learn what you have missed in the past* by ordering a computer **NTISearch** of all the research reports in your area of interest, dating as far back as 1964, if you wish. Please request NTIS-PR-186/PCN for more information.

WRITE: Managing Editor
5285 Port Royal Road
Springfield, VA 22161

Keep Up To Date With SRIM

SRIM (Selected Research in Microfiche) provides you with regular, automatic distribution of the complete texts of NTIS research reports *only* in the subject areas you select. SRIM covers almost all Government research reports by subject area and/or the originating Federal or local government agency. You may subscribe by any category or subcategory of our WGA **(Weekly Government Abstracts)** or **Government Reports Announcements and Index** categories, or to the reports issued by a particular agency such as the Department of Defense, Federal Energy Administration, or Environmental Protection Agency. Other options that will give you greater selectivity are available on request.

The cost of SRIM service is only 45¢ domestic (60¢ foreign) for each complete microfiched report. Your SRIM service begins as soon as your order is received and processed and you will receive biweekly shipments thereafter. If you wish, your service will be backdated to furnish you microfiche of reports issued earlier.

Because of contractual arrangements with several Special Technology Groups, not all NTIS reports are distributed in the SRIM program. You will receive a notice in your microfiche shipments identifying the exceptionally priced reports not available through SRIM.

A deposit account with NTIS is required before this service can be initiated. If you have specific questions concerning this service, please call (703) 451-1558, or write NTIS, attention SRIM Product Manager.

This information product distributed by

NTIS U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

167041

EPA-600/3-76-038
April 1976

PB 253 528

Ecological Research Series

[illegible] CYANIDES AND RELATED [illegible]

[illegible]

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-76-038	2.	3. RECIPIENT'S ACCESSION NO. PB-253 528
4. TITLE AND SUBTITLE TOXICITY TO FISH OF CYANIDES AND RELATED COMPOUNDS -- A REVIEW		5. REPORT DATE April 1976 (Issuing Date) 6. PERFORMING ORGANIZATION CODE N/A
7. AUTHOR(S) Peter Doudoroff		8. PERFORMING ORGANIZATION REPORT NO. N/A
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Fisheries and Wildlife Oregon State University Corvallis, Oregon 97331		10. PROGRAM ELEMENT NO. 1BA608 11. CONTRACT/GRANT NO. Grant R-802459
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Office of Research and Development Environmental Research Laboratory Duluth, Minnesota 55804		13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA-ORD
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

The world literature on the toxicity to fish of simple and complex cyanides, nitriles, cyanogen chloride, thiocyanates, and cyanates is reviewed critically and interpretively. Differently determined limits of toxicant concentrations tolerated by various fishes are compared, and their variation with exposure time, the pH, temperature, and dissolved oxygen and mineral content of the water, body size, age, acclimation, etc., is examined. Interactions of free cyanide with other toxic water pollutants also are considered. Available data on effects of sublethal levels of free cyanide on growth, food consumption and utilization, swimming ability, behavior, etc., and observations on avoidance reactions of fish to the toxicant are summarized and their ecological significance is discussed. After a brief introduction to the chemistry of complex metallocyanides and their behavior in dilute solutions, the acute toxicity of the solutions is thoroughly considered and related to concentrations of their identifiable components. The dominant role of molecular hydrocyanic acid produced by dissociation or photolysis of the metallocyanide complexes as a lethal agent responsible for the toxicity of most of the toxic solutions tested is given particular attention; the relative toxicity of complex metallocyanide ions also is considered. Some conclusions regarding acceptable concentrations of free cyanide in receiving waters are presented.

PRICES SUBJECT TO CHANGE

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Nitriles	Behavior	Fish toxicity	06F
Thiocyanates	Reviews	Cyanogen chloride	06S
pH	Toxicity	Exposure time	06C
Temperature	Cyanides	Swimming ability	
Growth	Interactions	Mettalocyanide	
Food consumption	Hydrogen cyanide	Concentrations acceptable	

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 161
	20. SECURITY CLASS (This page) Unclassified	22. PRICE 6.75

☆USGPO: 1976 — 657-695/5403 Region 5-11

EPA-600/3-76-038
April 1976

TOXICITY TO FISH OF CYANIDES AND RELATED COMPOUNDS

A Review

by

Peter Doudoroff
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon 97331

Grant No. R-802459

Project Officer

Donald I. Mount
Environmental Research Laboratory
Duluth, Minnesota 55804

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
DULUTH, MINNESOTA 55804

ia)

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

The world literature on the toxicity to fish of simple and complex cyanides, nitriles, cyanogen chloride, thiocyanates, and cyanates is reviewed critically and interpretively. Differently determined limits of toxicant concentrations tolerated by various fishes are compared, and their variation with exposure time, the pH, temperature, and dissolved oxygen and mineral content of the water, body size, age, acclimation, etc., is examined. Interactions of free cyanide with other toxic water pollutants also are considered. Available data on effects of sublethal levels of free cyanide on growth, food consumption and utilization, swimming ability, behavior, etc., and observations on avoidance reactions of fish to the toxicant are summarized and their ecological significance is discussed. After a brief introduction to the chemistry of complex metallocyanides and their behavior in dilute solutions, the acute toxicity of the solutions is thoroughly considered and related to concentrations of their identifiable components. The dominant role of molecular hydrocyanic acid produced by dissociation or photolysis of the metallocyanide complexes as a lethal agent responsible for the toxicity of most of the toxic solutions tested is given particular attention; the relative toxicity of complex metallocyanide ions also is considered. Some conclusions regarding acceptable concentrations of free cyanide in receiving waters are presented.

This report was submitted in fulfillment of Grant Number R-802459 by Oregon State University under the (partial) sponsorship of the Environmental Protection Agency. Work was completed as of September 1975.

CONTENTS

Sections Page

I Conclusions 1

II Introduction 4
  General 4
  Terminology and Analytical Methods 6
  Nomenclature 10

III Lethal Toxicity of Free Cyanide 12
  Influence of pH, and the Relative Toxicity of HCN and the CN Ion 12
  Results of Constant-Flow Tests (General) 14
  Results of "Static" Tests (General) 20
  Relations Between Cyanide Concentration and Survival Time 30
  Influence of Temperature 32
  Influence of Dissolved Oxygen 42
  Influence of Water Salinity and Hardness 45
  Effects of Acclimation to Cyanide 48
  Resistance in Relation to Body Size and Physiological State 50
  Interactions of Free Cyanide with Other Poisons 54
  Antagonistic Action of Thiosulfate 60
  Field Observations of Cyanide-Caused Fish Mortalities 61

Preceding page blank

Sections Page

IV Sublethal Toxicity of Free Cyanide and Avoidance Reactions of Fish 64
- Effects on Swimming Ability 64
- Effects on Growth, Food Consumption, and Food Utilization 67
- Effects on Embryonic Development, Respiration, and Heart Beat 70
- Tests for Other Nonlethal Injury 74
- Avoidance Reactions 78

V Toxicity of Complex Cyanides 83
- General, Chemical Background 83
- Toxicity of the Metallocyanide Complexes in General 90
- Zinc-Cyanide and Cadmium-Cyanide Complexes 96
- Nickel-Cyanide Complex 101
- Silver-Cyanide Complex 113
- Copper-Cyanide Complexes 120
- Iron-Cyanide Complexes 126

VI Toxicity of Other, Related Compounds 135
- Nitriles 135
- Cyanogen Chloride 140
- Thiocyanates 141
- Cyanates 143

VII References 144

SECTION I

CONCLUSIONS

The observed toxicity to fish of most of the tested, acutely toxic solutions of both simple and complex cyanides, and also those of acetaldehyde cyanohydrin (lactonitrile), is attributable very predominantly, or almost entirely, to the presence of molecular (undissociated) hydrocyanic acid, HCN, whose concentrations are reliably measurable. The toxicity of the cyanide ion, CN^-, which is a minor component of the so-called free cyanide ($HCN + CN^-$) in polluted waters that are not exceptionally alkaline, is indeterminable but of little importance. Also undetermined but clearly of no importance is the toxicity of zinc-cyanide and cadmium-cyanide complex anions, which are almost totally dissociated in very dilute but highly toxic solutions of the complexes. The acute toxicity of more stable silver-cyanide and cuprocyanide complex anions is much less than that of molecular HCN but is not negligible; these ions can be the principal toxicants even in some very dilute solutions. The much lower toxicity of the ferrocyanide and ferricyanide complex ions, which are complexes of high stability but subject to extensive and rapid photolysis, yielding free cyanide, on exposure to direct sunlight, and also of the nickelocyanide complex ion, is not likely ever to be of any practical importance.

The chronic toxicity of the metallocyanide complexes has not yet been investigated. One can reasonably conclude, nevertheless, that in the absence of important toxic pollutants other than simple and complex cyanides, the determined molecular HCN content of a cyanide-polluted water is a fairly reliable index or measure of its toxicity to fish, except under some rather unusual circumstances that generally can be readily recognized. The relatively high degree of dissociation of moderately stable cyanide complexes, such as the nickelocyanide complex, in exceedingly dilute solutions in which molecular HCN levels are low but are likely to have serious sublethal effects on sensitive fishes should be noted in this connection. Also to be borne in mind is the high toxicity of some of the metal ions. Total (free and complexed) cyanide concentrations are not toxicologically meaningful. In view of present availability of reliable analytical methods for the determination of low-level molecular HCN and free cyanide in polluted waters, there appears to be little justification for continued reliance only on total cyanide determinations in the evaluation and control of water pollution with cyanides.

Free cyanide or molecular HCN concentrations as low as 0.01 mg/l can rapidly and lastingly impair the swimming ability of salmonid fishes in well-oxygenated water. Lethal threshold concentrations may be as low as 0.02-0.025 mg/l at very low temperatures, though apparently they are generally above 0.05 mg/l under favorable conditions. The susceptibility of these and other fishes to cyanide poisoning is markedly increased at low oxygen concentrations. Clearly, free cyanide concentrations above 0.005 mg/l cannot be always entirely harmless to the salmonids and other very sensitive fishes, and only much lower levels may be truly safe concentrations in most waters in which such susceptible forms must be fully protected from any possible injury by toxicants.

On the other hand, there is, as yet, no evidence that even persistent free cyanide or HCN concentrations not far exceeding 0.025 mg/l, in waters not otherwise seriously polluted, are incompatible with the persistence of valuable fisheries. No conspicuous impairment by such concentrations of the swimming ability, growth, embryonic and larval development, behavior, etc., of relatively resistant species, for which the lowest experimentally determined lethal threshold concentrations in well-oxygenated water are well above 0.10 mg/l, has been demonstrated. Such resistant, warm-water species can be the species of major commercial or recreational importance inhabiting some waters subject to cyanide pollution, waters whose fisheries may not, for social and economic reasons, merit protection of the highest degree. Free cyanide concentrations as high as 0.05 mg/l seem to be too close to reported lethal threshold concentrations even for moderately resistant fishes and to sublethal levels clearly harmful to such species to be judged acceptable in any waters whose fisheries are to be afforded more than minimal protection.

The ecological significance of observed metabolic disturbances in fishes exposed to cyanide solutions and of the impairment of their swimming ability, as well as of avoidance reactions to the poison seen in laboratory tests, is still unclear. Much more information regarding the effects on fish of sublethal concentrations of cyanides than is now available, especially effects under nearly natural conditions, obviously is needed. Information concerning sublethal injurious effects of other, related compounds, such as cyanogen chloride, whose acute toxicity is about as great as that of free cyanide, and the typical nitriles, whose acute toxicity is less, but which, unlike free cyanide, evidently can be important accumulative poisons, is totally lacking.

SECTION II

INTRODUCTION

GENERAL

This is a comprehensive, critical, and interpretive review of the voluminous world literature on the toxicity to fish of the simple and complex cyanides and of related compounds, such as the nitriles, thiocyanates, and cyanogen chloride. Avoidance reactions of fish to the cyanides also are considered, together with other sublethal effects of these toxicants. A need for such a review has become increasingly apparent with increase in number of pertinent publications, in which some strikingly divergent or seemingly contradictory findings and evidently erroneous and misleading conclusions have been reported. Recently intensified efforts strictly to regulate, largely for the protection of fisheries, the disposal of industrial wastes containing cyanides have been hampered not only by a lack of much of the needed information, but also by frequent misunderstanding or ignorance of pertinent information already available. The present review should be helpful in this connection and also in the planning of additional research, which is certainly needed and may be stimulated by definition of the major, still unsolved or incompletely solved problems. Literature antedating 1915 is not considered.

Cyanides, whose careless discharge into public waters by electroplaters and others has in the past caused many disastrous fish mortalities, are still among the important water pollutants endangering fish and other aquatic life. They occur in waste waters from metal-finishing and metallurgical (e.g., gold and silver ore reduction) plants, steel mills, petroleum refineries, gas plants, and many other industrial sources. Improved waste disposal controls have reduced the frequency of sporadic, gross pollution of waters with cyanides (strong wastes, etc.) attributable simply to carelessness or ignorance, but less obvious, chronic pollution resulting from continuous discharges of waste waters with relatively low concentrations of the toxicants is still common. Definition of maximum concentrations of cyanides that are harmless to fish life or acceptable in receiving waters whose fish populations are to be protected so that fish production will not be seriously impaired is essential to sound waste disposal and water quality management wherever these toxic compounds can be important pollutants.

Unfortunately, most of the pertinent literature has to do with lethal effects and tolerance limits, and the available information on sublethal effects of cyanides is still scanty. Knowledge of the tolerance limits alone obviously is not a sufficient basis for any definite conclusions as to limits of entirely harmless concentrations of toxicants. A brief summary of the lethality data would surely suffice if this information could be useful only in explaining or preventing fish mortalities. However, comparison of such data obtained in tests with different species of fish or under varying experimental conditions reveals differences in susceptibility of the test animals to lethal effects of the toxicants that can be reasonably expected to be usually accompanied by similar differences in susceptibility to sublethal injury. And much of what has been learned recently about the relative acute toxicity of the different components of solutions of complex cyanides (i.e., about the causative agents of the rapidly lethal toxicity of these solutions) is surely pertinent to sublethal or chronic toxicity problems also. Results of experiments on the toxicity of the complex cyanides have been often misinterpreted in published literature, largely because of insufficient understanding of the complicated chemistry of dilute solutions of these compounds. Misunderstanding of the significance of these data has been reflected in illogical and confusing waste disposal regulations or effluent and water quality standards that have been proposed or adopted by regulatory agencies and can be either excessively or insufficiently restrictive or demanding under different circumstances. Widespread adoption of wastewater treatment that removes nearly all of the free cyanide renders increasingly important understanding of the toxicological properties and of the chemical behavior in receiving waters of any remaining cyanide complexes. For the above reasons, much attention is given in this review to some comparative lethality studies and especially to the available data on the acute toxicity of the complex cyanides and to their interpretation in chemical terms. It is my hope that future research in the sublethal toxicity of the cyanides and related compounds thus will be greatly facilitated.

TERMINOLOGY AND ANALYTICAL METHODS

In aqueous solutions of cyanides, the cyanide group, CN, can exist in different forms that must be distinguished. To avoid possible misunderstanding, the pertinent terminology used in this review must be carefully explained, with some attention to analytical methods that have been used for determination of cyanide in its different forms.

The designations cyanide ion and CN^- often have been used in published literature synonymously with free cyanide or with total cyanide, both of which terms will be defined presently. Such inappropriate and confusing terminology probably is responsible for the error to be found in the generally authoritative publication "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, et al., 1971), which attributes the toxicity of cyanide solutions to the formation in them of the cyanide ion, CN^-. Actually, for reasons to be fully explained later, the toxicity of cyanide-polluted waters to aquatic life is referable, as a general rule, mostly or entirely to molecular hydrocyanic acid, HCN, and not to the CN^- ion, although the latter form of cyanide probably is not without toxicity. In this review, the term cyanide ion is used in referring only to the simple, free anion CN^- *per se*, excluding all other cyanide species. In my opinion, this is the only strictly correct and toxicologically sound use of the term.

Hydrocyanic acid, HCN, ionizes in some degree in aqueous solutions, yielding cyanide ion, the extent of the ionization depending on the pH. That HCN which is not ionized but is present in the form of uncharged, intact molecules only, is usually best referred to explicitly as *molecular HCN*, hydrocyanic acid, or hydrogen cyanide to avoid misunderstanding (see the next paragraph), but here it is sometimes referred to simply as HCN for brevity where the intended meaning is clear enough.

The term *free cyanide* is used in referring to both the CN^- ion and the molecular HCN present in a solution, considered together and without regard to their sometimes multiple sources, which may be simple, alkali cyanides or metallocyanide complexes (complex anions) that dissociate or decompose in varying degrees. Only the weight of the cyanide group or radical, CN, usually is considered in reporting the free cyanide concentration in an experimental solution, but sometimes the equivalent weight of HCN has been reported instead, HCN being almost always the predominant component. The latter practice cannot be seriously misleading when the mole ratio of CN^- to HCN is very small, as it usually is, and it should be noted that the difference in molecular weight between HCN and CN is too small to be of any real consequence in this connection. The expression "free cyanide as HCN" signifies that CN^- is included.

Complexed cyanide is only that cyanide which is actually bound up in a complex or complexes and does not include that which has been liberated through dissociation or decomposition of a complex, forming HCN or CN^- ion.

The term total cyanide has been used in the literature in somewhat different senses. When used by chemists concerned with water quality, it usually embraces all cyanide species or groups determinable by a particular, selected analytical method involving acid distillation, in the course of which all or most of the complexed cyanide usually is liberated as HCN and trapped for measurement as CN^- ion. Although the term implies no exclusion, actually not all of the cyanide groups present in a sample may be included in the measurement, and values obtained for the same sample by different, widely used and approved procedures may differ considerably. Some cyanide not initially present may even be generated during the distillation. In this review, the term total cyanide is used as a designation for both free cyanide and complexed cyanide considered together and without regard to amounts recoverable or measurable by any analytical method. Thus, it is used in reporting amounts of cyanide as CN known to have been added to water in one form or another in preparing experimental solutions in which some of the cyanide was known to have been complexed. It may be qualified by words such as "initial", "added", or "introduced" when there are reasons for believing that a considerable portion of the added cyanide was or may have been lost to the atmosphere, destroyed by chemical reaction, or otherwise eliminated during or before a test of a solution for toxicity.

Standard analytical methods for the colorimetric determination of total cyanide in water are well known and have long been used extensively in connection with water pollution control, but they have only very limited applicability to toxicological research. On the other hand, only recently (i.e., in the past 15 years) has there been much interest and progress in the development of reliable and sufficiently sensitive analytical methods for the determination of free cyanide, molecular HCN, or CN^- ion in waters containing cyanide complexes of widely varying stability. Although several such methods have now been developed and published, they are still little known and apparently have been profitably employed in the water quality field only by a few investigators engaged in research in fish toxicology. There appears to have been a curious indifference to these methodological advances or resistance to their practical application, even though it has long been obvious that the total cyanide determinations made and reported routinely in the past are often insufficiently instructive, if not meaningless, as measures of water quality.

Most of the recently developed methods for determination of molecular HCN are essentially modifications of a crude colorimetric method published long ago by Worley and Browne (1917). Each involves distribution of the HCN between water (the sample) and dispersed air or nitrogen, trapping of the displaced HCN in one way or another, and its subsequent measurement as HCN or as CN^- ion in an alkaline solution. The final measurement is done by gas-liquid chromatography, using a thermal conductivity detector (Schneider and Freund, 1962) or, for increased sensitivity, a flame ionization detector (Claeys and Freund, 1968), polarographically (Nelson and Lysyj, 1971), or colorimetrically (Broderius, 1973). Accuracy of such methods depends on the use of water samples large enough and volumes of air or nitrogen small enough to ensure that only a very small fraction of the HCN initially present in each sample will be displaced and there will be no material change in pH of the sample due to displacement of carbon dioxide. Material disturbance of existing equilibria thus can be avoided. Somewhat different in principle is a method that involves colorimetric measurement of the transfer of HCN from a water sample to a sodium hydroxide solution by diffusion through air in a sealed container (Great Britain, Ministry of Technology, 1967; Brown, Shurben, and Shaw, 1970). Decidedly different is the methylchloroform extraction method described by Montgomery, Gardiner, and Gregory (1969), which is convenient to use in the field and appears to be sound in principle but has not been shown to be sufficiently reliable. For reasons not known, much disagreement between HCN concentrations found by the authors in solutions containing cyanide complexed with nickel and the corresponding, computed equilibrium levels of molecular HCN in these solutions has been noted (Broderius, 1973).

Cyanide ion and free cyanide concentrations in waters of known pH can be readily derived from determined levels of molecular HCN. Even the most refined electrochemical methods for determination of cyanide ion that have been recently developed probably do not have the sensitivity requisite for detection, in waters that have not been rendered highly alkaline, of the very low levels of free cyanide that often need to be measured.

NOMENCLATURE

Common names are used mostly in this review for species of fish that investigators have used as test animals, but scientific names also are given. Currently preferred or accepted common and scientific names are used in place of some of those given by authors whose findings are reported. A scientific name, once given, is not repeated when the same species, identified by common name, is mentioned more than once in the same paragraph or in successive paragraphs. Moreover, for a small number of species that have been much used as test animals, only the common names usually are given, for frequent repetition of the scientific names of these well-known species after their first mention is deemed unnecessary and would be wasteful of space. A list of the common and scientific names of these repeatedly mentioned species follows:

Bluegill	*Lepomis macrochirus*
Brown trout	*Salmo trutta*
Fathead minnow	*Pimephales promelas*
Guppy	*Poecilia reticulata* (*Lebistes reticulatus*)
Rainbow trout	*Salmo gairdneri*
Threespine stickleback	*Gasterosteus aculeatus*

SECTION III

LETHAL TOXICITY OF FREE CYANIDE

INFLUENCE OF pH, AND THE RELATIVE TOXICITY OF HCN AND THE CN^- ION

The distribution of free cyanide between its two different forms, that is, the mole ratio of the CN^- ion to molecular HCN, depends on the so-called hydrogen ion concentration (activity of hydronium ion, H_3O^+, commonly still designated for convenience by the symbol H^+). Pertinent equilibrium equations are:

$$\frac{[H^+]\,[CN^-]}{[HCN]} = K_a\,, \qquad \text{or} \qquad \frac{[CN^-]}{[HCN]} = \frac{K_a}{[H^+]}\,,$$

where K_a is the ionization constant of HCN, which increases markedly with increase of temperature and is about 4-6 x 10^{-10} at 20-25° C, and the bracketed symbols represent molar concentrations (or, strictly speaking, activities) of the indicated species or solution components. The exact value of $[CN^-]$ in any cyanide solution thus can be derived from a known value of [HCN], and vice versa, and both can be derived from their sum, a known concentration of free cyanide, if the pH of the solution, which is the negative logarithm of $[H^+]$, and the appropriate value of K_a are exactly known. At pH 8.3, which is not often exceeded in natural, surface waters, only about 9 percent (not more than 11%) of any free cyanide occurs as cyanide ion when the temperature of the water is between 20 and 25° C, and the percentage declines steeply with reduction of the pH, becoming only about 0.5 percent at neutrality (pH 7.0). Even at the quite unusual pH of 9.0, some two-thirds of the free cyanide is in the form of molecular HCN. Thus, even if the two forms of free cyanide were of equal toxicity, most or nearly all of the toxicity of waters polluted mainly with free cyanide would be usually due to their molecular HCN content and not correctly attributable to the presence of cyanide ion.

The acute toxicity to fish of solutions of simple alkali cyanides, or of free cyanide, has been found to decrease with increase of the pH of the solutions, and it has been concluded, therefore, that molecular HCN is more toxic than the CN^- ion. In a small series of tests performed by Wuhrmann and Woker (1948), the average time to lasting loss of equilibrium of the chub (cyprinid) *Squalius cephalus* in sodium cyanide (NaCN) solutions of the same cyanide content (0.66 mg/l as CN) and varying pH increased from about 52 minutes to 70 and 94 minutes with increase of pH from about 7.6-7.7 to 8.12 and 8.84, respectively. However, it declined again somewhat, to 73 minutes, with further increase of the pH to 9.5. The decrease of toxicity with increase of pH was attributed by the authors to the decrease of the concentration of molecular HCN, and the increased toxicity at the highest pH to the additional harmful action of the very high pH itself. Bridges (1958) reported that fish (largemouth bass, *Micropterus salmoides*, green sunfish, *Lepomis cyanellus*, and others) were more resistant to NaCN (1.0 mg/l as NaCN, or 0.53 mg/l as CN) at pH 8.4 and 8.9 in aquaria and at pH 9.7 in a pond treated with the chemical than they were at much lower levels of pH. In aquarium tests with only a few fish, largemouth bass lived for about 80 minutes at pH 8.9 but were killed in about 50 minutes, on the average, at pH 7.0-7.3. Fish began to come to the surface only about 30 minutes after application of the poison to the pond with pH 9.7 and within 15 minutes in a comparable pond with about the same surface temperature. Water temperatures were around 25° C in the aquarium tests and 17° C in the comparable ponds.

The above, limited observations cannot be said to be entirely conclusive. However, they are in agreement with some earlier observations on the toxicity of HCN to aquatic organisms other than fish, and also with observations on the toxicity to fish of salts of another weak acid, hydrogen sulfide, and the weak base ammonia in solutions of varying pH (Doudoroff and Katz, 1950). In each case, the undissociated or molecular form of the acid or base was found to be much more toxic than the acid anion or base cation. The greater toxicity of the molecular form is attributable to the relative ease with which small, uncharged molecules penetrate into the blood and other tissues of organisms, whose external membranes are relatively impermeable or less permeable to the charged ions. Internally, the chemically reactive cyanide ion must be the active agent of cyanide poisoning, just as the hydronium ion, which also does not penetrate living membranes readily, must be the active agent of poisoning of fish with carbon dioxide and carbonic acid (H_2CO_3), whose anion is harmless. Alexander, Southgate, and Bassindale (1935) reported that no effect of pH variations within the range of 6.0 to 8.5 on the toxicity of KCN solutions (0.3 mg/l as CN) to rainbow trout was seen. However, the failure to observe differences of overturning time in tests with limited numbers of fish may well have been due to insufficient change of the rapidly lethal molecular HCN content of test solutions with a change of pH within the experimental range at the low test temperature (7-8° C).

RESULTS OF CONSTANT-FLOW TESTS (GENERAL)

The acute or more or less rapidly lethal toxicity of simple alkali cyanides to fish at ordinary experimental temperatures and under otherwise favorable conditions has been studied by a large number of investigators. Free cyanide is not a persistent toxicant, and so is lost fairly rapidly from unrenewed, standing test solutions in which fish are held. Therefore, the results of only those fairly prolonged toxicity tests during which the test solutions are continuously renewed (i.e., so-called constant-flow or continuous-flow bioassays) can be entirely reliable. Such tests, with complete immobilization or permanent overturning (loss of equilibrium) of the fish taken as the end-point of "survival", have been performed only with a rather small number of fish species of limited variety. The duration of a number of these tests was as long as 3 days or much longer, but others lasted for only about 1 day or less. Average or median periods of survival of the fish at tested concentrations of the toxicants, or percentages of test animals surviving at these concentrations for various, sometimes quite prolonged, exposure periods have been determined. From recorded survival percentages, median tolerance limits (median lethal concentrations, or toxicant concentrations at which just 50 percent of the test animals are able to survive) for the periods of actual exposure of the fish to test solutions have been derived by interpolation. Also, lethal threshold concentrations (incipient lethal levels, or concentrations nearly or barely tolerable for individuals of average resistance when exposure thereto is indefinitely prolonged) often have been estimated by extrapolation, i.e., derived from observed relationships between concentration and average or median survival time, using graphical or computational methods believed to be appropriate. Some of the estimates (indicated threshold levels) have been based on results of tests of rather short duration only. Most of the estimated lethal threshold concentrations and reported or indicated median tolerance limits for exposure periods of about 3 days or longer have been found to fall within the range of 0.05 to 0.16 mg/l as CN when results of constant flow tests only were considered, but a few of the values are well below or above this range. Potassium cyanide (KCN) was used in most of the tests.

Karsten (1934) reported that all of eight brook trout, Salvelinus fontinalis, about 15 cm long died within 136 hours at a free cyanide concentration (in KCN solutions) of 0.05 mg/l as CN, and all of six survived for 27 days at 0.02 mg/l as CN. However, Neil (1957) found a KCN solution with a concentration of 0.05 mg/l as CN (amount of cyanide added to water) to be nonlethal to brook trout 10-13 cm long in an exposure period of 40 days at least; only five of ten brook trout had turned over permanently after 87 hours at a concentration of 0.08 mg/l as CN. These results agree well with those obtained with young rainbow trout, Salmo gairdneri, by Herbert and Merkens (1952), who reported an average survival time of 74 hours at a KCN concentration of 0.07 mg/l as CN. They agree also with results obtained in experiments with young brown trout, Salmo trutta, by Burdick, Dean, and Harris (1958), who estimated the lethal threshold concentration for these fish at high oxygen concentrations to have been about 0.09 mg/l as CN, and who observed only a 40 percent mortality after 10 days in one test at a KCN concentration of 0.07+ mg/l as CN. Test temperatures were near 9.5° C in Neil's experiments, 17.5° in those of Herbert and Merkens, whose rainbow trout averaged a little less than 10 cm in length, and about 15.5° C in the experiments of Burdick, Dean, and Harris, who tested brown trout averaging less than 5 cm and about 10 cm in length with quite similar results; the water temperature in Karsten's experiments is unknown.

At a test temperature of 12-13° C, more recent experiments with young rainbow trout reported by British investigators (Great Britain, Ministry of Technology, 1968) have yielded results closely agreeing with the above-mentioned data on the cyanide tolerance of the same and other kinds of trout. A lethal threshold concentration of free cyanide in the vicinity of 0.08 mg/l as CN (between 0.08 and 0.09 mg/l as HCN) seemed to be indicated. However, when tests were performed at much lower temperatures near 3° C, the results indicated a lethal threshold level between 0.02 and 0.025 mg/l. Additional tests performed later also demonstrated greatly increased sensitivity of the trout to free cyanide at very low temperatures. Thus, 24-hour median lethal concentrations for rainbow trout acclimated for 7 days to a temperature of 3° C averaged about 0.04-0.05 mg/l (Great Britain, Department of the Environment, 1972), suggesting that the lethal threshold concentration for these fish was probably below 0.03 mg/l. These interesting results will be more fully considered later, in connection with a general discussion of the influence of temperature on resistance to cyanide. Data on the adverse effects of low dissolved oxygen concentrations also will be reviewed in detail later. Burdick, Dean, and Harris (1958) observed only a moderate effect of reduction of the oxygen concentration to 5.2 mg/l on the cyanide tolerance of their brown trout at about 15.5° C. Under these conditions, the lethal threshold concentration was estimated by them to have been about 0.08 mg/l as CN.

The lethal threshold concentration of cyanide for smallmouth bass, Micropterus dolomieui, averaging about 6.3 cm in length, in KCN solutions at a temperature of 21° C, was estimated by Burdick, Dean and Harris (1958) to have been about 0.104 mg/l as CN at high oxygen concentrations, but near 0.086 mg/l at the moderately reduced dissolved oxygen concentration of 4.2 mg/l. At the high oxygen concentrations, the 23-hour median tolerance limit was 0.127 mg/l; this cyanide concentration did not kill all of the test animals in 3 days. Doudoroff, Leduc, and Schneider (1966) found the 48-hour and 72-hour median tolerance limits (50 percent lethal concentrations) of NaCN for young bluegills, Lepomis macrochirus, at 20° C to be nearly equal, about 0.16 mg/l as HCN (0.154 mg/l as CN). Of eight bluegills that survived for 48 hours at test concentrations of 0.155 and 0.180 mg/l as HCN, from a total of 20 fish averaging about 5 cm in length that were tested at these two concentrations, only one died during the following day; the remaining seven were apparently healthy after the 72-hour exposure. Lipschuetz and Cooper (1955) reported 0.22 mg/l as CN to have been the 24-hour median tolerance limit of KCN for blacknose dace, Rhinichthys atratulus meleagris, about 3.8-7.6 cm in length at temperatures around 21° C and high oxygen concentrations.

Brockway (1963) found the 24-hour and 48-hour median tolerance limits of NaCN at 25° C for juvenile cichlids, Cichlasoma bimaculatum, 4-5 months old and weighing about 1.0-1.3 g, to be about 0.18 and 0.135 mg/l as CN, respectively. In a prolonged test with young of the same species initially about 2 months old and averaging about 0.5 g in weight, no deaths were observed during a 60-day exposure of the fish to a concentration of 0.11 mg/l as CN. This species of aquarium fish has been much used in studies of sublethal effects of cyanide poisoning to be considered later. The highest long-term tolerance limit of free cyanide for fish that has been evaluated by the constant-flow bioassay technique and reported in published literature apparently is the theoretical lethal threshold concentration for guppies, Poecilia reticulata (Lebistes reticulatus), 1 month old at 24-24.5° C estimated by Chen and Selleck (1969), namely, 0.236 mg/l as CN in solutions of KCN. Anderson (1974), however, found the 96-hour median tolerance limit of KCN for small, adult, male guppies about 0.1 g in weight at 25° C to be about 0.147 mg/l, doubtless as CN, although reported (erroneously, I am sure) as a concentration of KCN. And the median survival time of guppies at a free cyanide concentration of 0.20 mg/l as CN and 18° C, probably in a constant-flow test (bioassay method not stated), had been previously reported to have been about 80 hours (Great Britain, Department of Scientific and Industrial Research, 1956). The latter result was one obtained in a comparative study of the relative resistance to free cyanide of ten species of fish, of which the guppy proved to be by far the most resistant one. Abram (1964) estimated a lethal threshold concentration of but 0.071 mg/l as CN for the Harlequin fish, Rasbora heteromorpha (another aquarium fish); the test conditions were not specified, but it seems reasonable to assume that the test solutions were continuously renewed.

The data reported by Renn (1955), obtained also by the constant-flow bioassay technique, are of limited value and not easily summarized and compared with the data reported above, because of the manner of their presentation (mostly graphical), as well as the relatively short duration of exposure to the test solutions of the several species of warmwater fish tested. Renn stated that 100 percent survival for 10 hours of the white crappie, Pomoxis annularis, proved uncertain (at 25° C) at KCN concentrations exceeding 0.03 mg/l as N (0.056 mg/l as CN), and that such survival of none of the tested species was observed at concentrations exceeding 0.06 mg/l as N (0.11 mg/l as CN). He also found that 50 percent mortality of bluegills and of redbreast sunfish, Lepomis auritus, often occurred at KCN concentrations as low as 0.11 to 0.14 mg/l as CN after exposure periods of only about 150 to 350 minutes (at 25° C). These concentrations are lower than the long-term median tolerance limit (of NaCN concentration at 20° C) for the bluegill estimated by Doudoroff, Leduc, and Schneider (1966), about 0.154 mg/l as CN.

Reported cyanide concentrations commonly have been amounts of cyanide added to the experimental water, and not amounts found by chemical analysis to have been actually present. When chemical analyses of the test solutions have been made and the results reported, for comparative purposes, together with the expected (computed) cyanide concentrations based on amounts of cyanide added, the analytically determined values generally have been shown to be, for some undetermined reasons, somewhat lower than the expected concentrations. Estimates of tolerance limits thus may be often a little too high. For example, Herbert and Merkens (1952) found the analytically determined cyanide concentration in the water to which 0.07 mg/l of cyanide (as CN) had been added and in which their rainbow trout survived for 74 hours, on the average, to be only about 0.06 mg/l. Likewise, Doudoroff, Leduc, and Schneider (1966) found that the molecular HCN concentrations in fresh, standing NaCN solutions like those used in their long-term, constant-flow, toxicity tests, determined by the analytical method of Schneider and

Freund (1962), were less than the expected (computed) concentrations by about 8 percent. The correct 48-hour and 72-hour median tolerance limits of free cyanide for their bluegills thus may have been near 0.14 mg/l as CN. Cairns and Scheier (1968), who performed "static" bioassays only (i.e., tests without continuous renewal of test solutions), also reported initial cyanide concentrations determined by analysis of their solutions to have been usually, but not always, decidedly lower than the expected concentrations computed from the amounts of cyanide added to their synthetic dilution water (i.e., a water prepared from distilled water by adding various chemicals). Analytical errors may well have been responsible, of course, for some of the differences in question. Burdick, Dean, and Harris (1958) and Renn (1955) stated that their test solutions were analyzed for cyanide, and their reported test concentrations may well have been based on the results of these analyses. No comparisons of the analytically determined concentrations with expected or computed concentrations were reported by these authors. Neil (1957) and Lipschuetz and Cooper (1955) analyzed some of their test solutions, but chose to rely on computed cyanide concentrations in reporting their test results.

RESULTS OF "STATIC" TESTS (GENERAL)

Because cyanide concentrations in unrenewed test solutions decline fairly rapidly, tolerance limits determined by so-called "static", or standing water, toxicity bioassays with no renewal of test solutions tend to be somewhat higher than those determined by constant-flow tests of equal, fairly prolonged duration. However, disagreement of the results even of quite prolonged bioassays of the two kinds generally has not been very great. Probably the main reason is that cyanide is a fairly rapidly acting poison; consequently, the relation between free cyanide concentration and survival time is such that maximum constant concentrations tolerated for long periods, or indefinitely, are not much lower than those just tolerated for periods of only 10 to 24 hours. Acclimation of surviving fish exposed to critical levels of the poison may be partly responsible for long or indefinite survival of these fish at concentrations that had proved rapidly lethal to other individuals, or at only slightly lower concentrations, even in constant-flow tests.

Review of the many results of determinations of the toxicity of free cyanide to fish by simple, static bioassay that are to be found in the literature does reveal some published tolerance limits that are inordinately high and grossly misleading. For example, Dorier (1952) has reported that a sodium cyanide concentration of 1/200,000 (i.e., 5 mg/l as NaCN, or about 2.7 mg/l as CN) is lethal to brown and rainbow trout, and a concentration half as great is dangerous for them. And concentrations four times as great, 1/50,000 and 1/100,000 (i.e., 10.6 and 5.3 mg/l as CN) were said to be lethal and dangerous, respectively, for the roach, Rutilus rutilus. Concentrations as high as 1/500,000 (about 1.1 mg/l as CN) and 1/200,000 (2.7 mg/l as CN) then were indicated to be harmless to the trout and the roach, respectively. These and other such underestimates of the toxicity of free cyanide must be attributed to faulty experimental techniques or, in some cases, to insufficiently prolonged exposure of the fish to test solutions, I suppose.

On the other hand, there are also some published reports of toxicity of free cyanide to certain fishes that is much greater than that observed by several other investigators. For example, Ellis (1937) reported that KCN concentrations of only 0.1 to 0.3 mg/l (0.04 to 0.12 mg/l as CN) in hard water killed goldfish, Carassius auratus, in 3 to 4 days. Other pertinent data in the available literature indicate that the goldfish is among the fishes least susceptible to cyanide poisoning. Powers (1917) reported that a 0.12×10^{-4} M solution of KCN, or about 0.31 mg/l as CN, killed goldfish in about 2 to 5 days at 21.5° C. And Costa (1965), who found the 50-hour tolerance limit of NaCN for each of four other fish species tested by him to be not much greater than 0.15 mg/l as CN, surprisingly reported a corresponding tolerance limit for the goldfish (at 17-18° C) greater than 1.5 mg/l as CN (above 6×10^{-5} N). At least, that is what is shown in his graph; his statement, on page 58, that "young goldfish survive...in a solution of 0.00001 N for more than 3 days" is not contradictory but is somewhat incongruous, or not fully in accord with the data presented graphically. His Figure 6 also is a little confusing, because the value 5×10^{-5} appears on each scale of abscissas (concentrations) where the value 5×10^{-6} obviously belongs instead. Bridges (1958) found that NaCN at a concentration of 1.0 mg/l (0.53 mg/l as CN) killed some of his goldfish only after an exposure of about 2 days and 0.5 mg/l killed none in 72 hours at 24-28° C. No explanation can be offered for the low lethal levels reported by Ellis, except the possibility of error in the preparation of test solutions or of serious, undetected depletion of dissolved oxygen in the solutions.

Fish embryos and young larvae have been found to be far more resistant to free cyanide than are the fully developed animals. For example, Cairns, Scheier, and Loos (1965) have reported that the 48-hour median tolerance limit of KCN for "eggs" (embryos) of the zebra danio, Brachydanio rerio, was estimated to have been about 11.7 mg/l as CN at temperatures near 24° C, whereas the reported, corresponding value for adults of the same species was 0.49 mg/l as CN. None of the eggs became opaque after exposure for 48 hours to a KCN concentration of 10 mg/l as CN. The embryos tested were at a fairly early stage of development; although all controls developed eye pigmentation within 24 hours after the beginning of the test, the embryos exposed to the tolerated, high concentrations of cyanide, which evidently inhibited their development, did not. Brinley (1930) found that the hearts of 6-day old embryos of the mummichog, Fundulus heteroclitus, exposed at an unspecified temperature to a M/160 solution of KCN in sea water (162 mg/l as CN) stopped beating only after about 27 hours. Karsten (1934) reported that a free cyanide concentration of 3.2 mg/l as CN, which killed brown trout 20-23 cm long within 7 minutes, was tolerated for several hours with no evident harmful effect by brown trout sac fry only a day old. He stated that unhatched and partially hatched trout eggs hatched successfully and the resulting fry remained alive at this cyanide concentration. However, Philips (1940) found that embryos of the cunner, *Tautogolabrus adspersus*, a marine fish which may be exceedingly susceptible to cyanide poisoning, could not tolerate a concentration of NaCN as high as 0.65 mg/l as CN; a concentration half as great was tolerated for a 27-hour observation period.

Most of the reported tolerance limits of free cyanide for juvenile and adult fishes exposed to the poison for about 1 day or longer (i.e., excluding values that pertain to much shorter exposure periods) that have been determined by static toxicity bioassays apparently without any renewal of test solutions fall within the range of 0.1 to 0.3 mg/l as CN. Such tolerance limits falling within this range have been reported for many common cyprinids, such as the fathead minnow, *Pimephales promelas* (Doudoroff, 1956; Henderson, Pickering, and Lemke, 1961), the European minnow, *Phoxinus phoxinus* (Costa, 1965), and the Asiatic, carp-like mrigal, *Cirrhina mrigala* (Seth, *et al*., 1967), for some generally hardy forms such as young eels, *Anguilla japonica* and *A. anguilla* (Oshima, 1931; Costa, 1965), for various centrarchids, such as the green sunfish, *Lepomis cyanellus*, (Lewis and Tarrant, 1960) and the bluegill, which has been used in many experiments whose results will be summarized and compared presently, for brown trout young and three-spine sticklebacks, *Gasterosteus aculeatus*, (Costa, 1965) and for many other kinds of fish.

Tolerance limits for warm-water fishes well below 0.1 mg/l as CN, evaluated by static bioassays, have been reported occasionally. Daugherty and Garrett (1951) found the 24-hour median tolerance limit of HCN (actually free cyanide as HCN) for the marine pin perch, *Lagodon* rhomboides, to be only about 0.07 mg/l, even though their test solutions, at uncontrolled temperatures ranging from 13.7 to 20.4° C, were continuously aerated with compressed air during the tests. Malacea (1966) reported the minimal lethal concentration of KCN for the cyprinid fish Leucaspius delineatus to be 0.06 mg/l as CN, a concentration which, according to his table of test results and an accompanying graph, actually killed these fish in about 2.5 hours at 19.5° C and pH 7.5. However, Gillar (1962), who performed 72-hour static tests, reported the tolerance limit of KCN for Leucaspius delineatus to be below 0.3 mg/l, a concentration at which 100 percent of the fish died, but well above 0.14 mg/l as CN, a concentration at which he observed no evident reaction of the fish to the presence of the poison. Thus, he found this fish to be less sensitive than the roach, Rutilus rutilus, for which, at the same test temperature (12° C), he determined the average lethal concentration limit to be 0.145 mg/l under the conditions of his tests. On the other hand, he found the lethal concentration limit for the percid fish Acerina cernua (ruffe) to be less than 0.1 mg/l as CN, a concentration at which all of these fish died in the test. And Woker and Wuhrmann (1950) estimated lethal threshold concentrations of HCN to be about 0.06 mg/l for the minnow Phoxinus laevis and about 0.08 mg/l for the perch Perca fluviatilis (but 0.10 mg/l for the hardy tench, Tinca vulgaris, and 0.30 mg/l for the chub Squalius cephalus). These estimates were derived by extrapolation, according to an equation deemed appropriate, probably from results of tests of relatively short duration, and so should not be regarded as proven thresholds. Such values tend to be lower, of course, than observed limits of tolerance for exposure periods that are not very long.

Lethal concentration limits of free cyanide well above 0.3 mg/l as CN have been recorded not infrequently for resistant species of fish, even when the duration of the tests on which the values were based was as long as 1 day or longer. For example, Wallen, Greer, and Lasater (1957) reported that the 24-hour median tolerance limit of KCN for the mosquitofish, Gambusia affinis, in a turbid water at 21-23° C was about 1.6 mg/l (0.64 mg/l as CN); the 96-hour tolerance limit was found to be the same. Schaut (1939) reported data indicating a 24-hour median tolerance limit of NaCN near 0.75 mg/l (0.40 mg/l as CN) for unidentified fresh-water "minnows (killies)." Wells (1916) found that a 1/25,000 N solution of KCN (1.04 mg/l as CN) killed the hardy black bullhead, Ictalurus melas, only after an exposure of almost 28 hours at an unspecified temperature, although other species of fish were killed rapidly (in about 1 hour or less) at this concentration. Nehring (1964) reported that yearling carp, Cyprinus carpio, about 11 cm long and weighing about 50 g, exposed to KCN solutions at 16° C, were evidently affected at concentrations of 0.4 mg/l as CN or more, but died within 3 days only at a concentration of 0.6 mg/l. This report of high resistance of young carp to cyanide poisoning is not unique, but Silaichuk (1969) observed death of 40 percent within 24 hours at a concentration of only 0.1 mg/l as CN.

With the exception of two reports already mentioned and one of a test in which loss of cyanide from the test solution was known to have been rapid (Kariya et al., 1967), I have found no published reports of ability of fully developed fish to survive for reasonably prolonged exposure periods in solutions with initial free cyanide concentrations much above 1.0 mg/l as CN. Even 0.3 mg/l may not be truly a nonlethal concentration for any fish species. But there is much evidence that some resistant warm-water species can live at free cyanide levels four or five times as great as levels that are lethal to other, more sensitive species (exclusive even of the cold-water salmonids) under essentially the same conditions. The large apparent differences in resistance among fish of various kinds tested by different investigators can be reasonably attributed only in part to variation of experimental methods and conditions, to which some of the noted, striking discrepancies of test results clearly must be ascribed. Large interspecific differences have been reported by some investigators who have tested several or numerous species for comparative purposes, presumably under quite similar conditions. Some of the comparable but very different results obtained with different species by the same investigators (Costa, 1965; Woker and Wuhrmann, 1950) already have been mentioned. Costa's goldfish apparently tolerated for 50 hours a free cyanide concentration about 10 times the maximum concentration tolerated by the more susceptible warm-water fishes used in his experiments. In Malacea's (1966) experiments, in which the cyprinids Leucaspius delineatus apparently were killed in about 2.5 hours by only 0.06 mg/l as CN (as already noted), Phoxinus phoxinus were killed in about 5 hours by 0.20 mg/l, and the bitterling, Rhodeus sericeus amarus, only in about 8 hours by 1.16 mg/l, at temperatures ranging from 18.5 to 22° C and pH 7.5-7.9. In other tests, median resistance periods at a cyanide concentration of 0.20 mg/l as CN and 18° C are reported to have been less than 3 hours for the rainbow trout, about 4, 8, and 12 hours for the Harlequin fish, Rasbora heteromorpha, the minnow Phoxinus phoxinus, and the zebra danio, Brachydanio rerio, respectively, and about 80 hours for the guppy, whose median resistance time at a concentration of 1.0 mg/l was well over 4 hours (Great Britain, Department of Scientific and Industrial Research, 1956). The nature of the tests (i.e., the method employed) is unknown, but constancy of cyanide concentrations is suggested by the relations between the tested concentrations and the median resistance periods presented graphically. In an unpublished report of an early investigation (Michigan Department of Conservation, Institute for Fisheries Research, 1933), in which many species were tested, the following "toxicity thresholds" of NaCN, here converted to equivalent cyanide concentrations as CN, were reported, among others: 0.53 mg/l as CN for adult mudminnows, Umbra limi, 0.53 mg/l or less for young carp; 0.39 mg/l for mottled sculpin, Cottus bairdi, adults; 0.265 mg/l for yellow bullhead, Ictalurus natalis, fingerlings and for half-grown pumpkinseeds, Lepomis gibbosus; 0.175 mg/l for rainbow darter, Etheostoma caeruleum, adults, for half-grown grass pickerel, Esox americanus vermiculatus, and for rock bass, *Ambloplites rupestris*, fingerlings; 0.155 mg/l for largemouth bass, *Micropterus salmoides*, fingerlings; and 0.11 mg/l for rainbow trout fingerlings.

Losses of free cyanide at different rates from unrenewed test solutions doubtless have been responsible for much of the variability of static test results obtained by different investigators, as well as the disagreement of results obtained by different methods. Doudoroff (1956) observed that an NaCN solution that killed all of his fathead minnows within 14 hours when it was fresh killed only 60 percent of minnows introduced after removal of the dead fish and when the solution was 24 hours old. Minnows introduced into the same solution when it was 96 hours old and the fish surviving in it had been removed were not noticeably affected at all. Bridges (1958) reported a number of similar experiments with green sunfish, *Lepomis cyanellus*; in these experiments, no fish apparently were placed in the aging solutions before the solutions were tested for toxicity. The solutions, with an initial NaCN content of 1.0 or 1.5 mg/l (0.53-0.80 mg/l as CN), killed all the test fish after aging for 24 hours, but killed none after aging for 72 hours in some experiments and only 48 hours in others. The depth of the test solution in Doudoroff's experiment was about 18 cm; that in Bridges' experiments was not stated. Cairns and Scheier (1963, 1968) determined initial and residual cyanide concentrations in their unrenewed test solutions, which probably were continuously oxygenated by mild, controlled aeration. They stated that the losses of analyzable cyanide during the 96-hour experimental period varied from 16 to 62 percent of the initial concentration (Cairns and Scheier, 1963). However, their tabulated data seem to show 96-hour losses frequently as great as 87.5 percent and always in excess of 40 percent. The determinations of the very low residual levels admittedly were not very reliable or precise. Kariya *et al*. (1967) observed a very rapid decline of cyanide concentration in their evidently overloaded test aquaria.

Burdick, Dean, and Harris (1958) compared results of parallel static and constant-flow toxicity bioassays of KCN, using brown trout as test animals. Although only rather high, rapidly lethal concentrations were tested, which killed all the fish in less than 4 hours, there was a pronounced and statistically quite significant difference of the results obtained by the two methods at the lowest concentration. In the constant-flow test, the geometric mean resistance time was only 30 minutes, whereas in the static test it was found to be more than twice as long with the same initial cyanide concentration. Even if the difference of mean resistance periods was fortuitously somewhat exaggerated, as it well may have been in this single test, it obviously can become very important when concentrations tested are much lower and the mean or median resistance periods under constant-flow test conditions are as long as 24 hours or longer, requiring correspondingly extended tests. Were it not for the already mentioned nature of the relationship between cyanide concentration and the survival time of fish, the results of prolonged static tests without frequent renewal of test solutions would have been much more misleading than they usually have been actually. Estimates of lethal threshold concentrations derived by extrapolation from such results obviously cannot be very reliable. By increasing susceptibility to cyanide poisoning, the reduction of dissolved oxygen concentrations in unaerated, standing test solutions with fish doubtless has tended to compensate somewhat for the loss of cyanide from the unrenewed solutions in static tests. But how low would have been the median tolerance limits of free cyanide determined for such very susceptible fishes as the marine pin perch, Lagodon rhomboides, had the constant-flow bioassay method been used and the tests continued for at least 48 or 96 hours? The 24-hour value for the pin perch reported by Daugherty and Garrett (1951), less than 0.07 mg/l as CN, was determined by a static bioassay in which the test solutions were aerated continuously. Yet it is as low as, or lower than, 3-day tolerance limits and even lethal threshold concentrations for salmonid fishes indicated by results of constant-flow tests under favorable conditions. A need for more and better experiments with fishes apparently (or possibly) much less resistant to cyanide poisoning than the salmonids is surely indicated.

The results of numerous tests of the toxicity of free cyanide to the bluegill have been fairly uniform. Cairns and Scheier (1958, 1959, 1963, 1968) and Patrick, Cairns, and Scheier (1968) reported 96-hour median tolerance limits of KCN for the bluegill determined with fish of various sizes in different dilution waters at temperatures of 18 or 20° C. These values, obtained by static bioassays with controlled aeration of test solutions, range from about 0.17 to about 0.23 mg/l as CN, and they pertain to amounts of cyanide added to the waters used, and not to analytically determined concentrations. It can be seen that they are not very much higher than the 48-hour and 72-hour median tolerance limits for the bluegill at 20° C (about 0.154 mg/l as CN) that have been determined by continuous-flow bioassay (Doudoroff, Leduc and Schneider, 1966) and that also pertain to cyanide added (but in the form of NaCN). It must be noted here that the 48-hour and 72-hour median tolerance limits reported by Cairns and Scheier (1963) for the 18° C temperature evidently are incorrect because of faulty interpolation. However, the experimental data needed for their correction are fully presented in tables, and the corrected values all fall within the range indicated above for 96-hour tolerance limits reported by the authors. Turnbull, DeMann, and Weston (1954) found both the 24-hour and the 48-hour median tolerance limits of KCN for the bluegill, determined by static bioassay at 20° C, to be about 0.28 mg/l as CN, a value apparently much too high, especially for the 48-hour limit. On the other hand, Broderius (1973) reported a median survival time of only 11 hours at a cyanide concentration (initial) of 0.15 mg/l as CN in a standing solution of NaCN at 20° C. Henderson, Pickering, and Lemke (1961) reported a 96-hour median tolerance limit of NaCN for bluegills, also determined by static bioassay without renewal of test solutions at the higher temperature of 25° C, of 0.15 mg/l as CN. A "toxicity threshold" of about 0.175 mg/l as CN (0.33 mg/l as NaCN) also has been reported (Michigan Department of Conservation, Institute for Fisheries Research, 1933). At the rather high temperature of 30° C, Cairns and Scheier (1963) found 96-hour median tolerance limits of KCN for the bluegill to be about 0.13 to 0.14 mg/l as CN, whereas comparable values determined in the course of the same study at 18° C were about 0.17 to 0.18 mg/l as CN. It should be noted that none of the above tolerance limits for the bluegill is much more than twice the lowest value reported, and all but one are less than twice the lowest value, even though the experimental methods and temperatures were not at all uniform. This agreement of the bioassay results is not any better, certainly, than the agreement to be expected among results of truly meaningful toxicity bioassays properly performed, at least when temperatures are fairly uniform. However, it compares most favorably with the agreement, or lack of agreement, of the published results of toxicity tests with some other species of fish used as test animals, such as the goldfish, Carassius auratus, for which 4-day tolerance limits of free cyanide ranging from less than 0.1 mg/l as CN (0.04-0.12 mg/l) to more than 1.5 mg/l have been reported, the brown trout, etc. The vast discrepancies of some of the findings reported in the published literature are all but inexplicable; gross methodological or other errors must be assumed in proposing plausible explanations.

RELATIONS BETWEEN CYANIDE CONCENTRATION AND SURVIVAL TIME

The relationships between free cyanide concentration and the survival time of fishes or the overturning time, and also the relationships between the duration of exposure to given cyanide concentrations and the percentage of test animals surviving, have been studied and discussed by a number of investigators (Wuhrmann and Woker, 1948; Woker and Wuhrmann, 1950; Wuhrmann, 1952; Herbert and Merkens, 1952; Herbert and Downing, 1955; Herbert, Downing, and Merkens, 1955; Neil, 1957; Burdick, 1957; Burdick, Dean, and Harris, 1958; Gillar, 1962; Abram, 1964; Doudoroff, Leduc, and Schneider, 1966; Chen and Selleck, 1969). Various equations describing the relationships mathematically have been advanced. These are pertinent to the estimation of lethal threshold concentrations that have been reported here, but a detailed and necessarily involved exposition of this matter would not be appropriate to the intended purpose of this literature review.

Some of the available data suggest a rectilinear relationship between the logarithms of cyanide concentrations and logarithms of mean or median resistance times over a wide range of cyanide concentrations (Herbert and Merkens, 1952; Neil, 1957; Gillar, 1962; Doudoroff, Leduc, and Schneider, 1966). Great deviations from this relationship at very high, rapidly fatal cyanide concentrations have been obvious whenever such concentrations have been tested. The existence of a minimum (threshold) response or resistance time has been assumed by some, as can be seen in the well-known equation:

$$(C - a)^n (T - b) = K$$

where C = concentration of the toxicant,
a = threshold concentration for response (e.g., lethal threshold or incipient lethal level),
T = duration of exposure (response or resistance time),
b = minimum or threshold response (or resistance) time,
and n and K are appropriate constants (Wuhrmann, 1952; Warren, 1971).

At the lowest concentrations tested, which were tolerated by rainbow trout for more than 3 days, the data of Herbert and Merkens (1952) revealed to them no deviation from the above-mentioned rectilinear relationship between the logarithms of concentration and of exposure time ($C^n T = K$, where the terms are as just defined above). There can now be no doubt, however, that lethal threshold concentrations of cyanide do exist (i.e., are real). The existence of a threshold level is clearly indicated even by some data that also have strongly suggested a linear relation between logarithms of median survival times and toxicant concentrations over wide ranges of these variables, as noted by Doudoroff, Leduc, and Schneider (1966). One can reasonably question only the accuracy of estimates of the threshold values that have been variously arrived at by extrapolation from relatively short-term lethality data, and perhaps debate the relative merits of estimation procedures that have been employed.

Herbert and Downing (1955) made the interesting observation that the frequency distribution of the periods of survival of individual rainbow trout in KCN solutions was symmetrical and approximately normal at rapidly lethal, high concentrations of the poison only. It was asymmetrical and approximately log-normal within that range of lower concentrations where the relation between log concentration and log survival time was believed to be linear, and not at concentrations well above that range. The significance of this observation is not clear.

INFLUENCE OF TEMPERATURE

Pronounced acceleration of the lethal action of free cyanide on fish due to increases of temperature has been observed by a number of investigators when various fishes have been exposed to constant, more or less rapidly lethal cyanide concentrations at the different temperatures. Linear relationships have been observed between temperature and the reciprocal of the overturning time (Southgate, Pentelow, and Bassindale, 1932; Alexander, Southgate, and Bassindale, 1935) or, more often, the logarithms of the overturning or final immobilization (lethal exposure) time or of its reciprocal (Wuhrmann and Woker, 1953, 1955; Sumner and Doudoroff, 1938). Nearly twofold to nearly threefold increases of the rate of lethal action of the poison with each 10° C rise of temperature (i.e., Q_{10} values of 1.8 to 2.8) have been recorded. It should be noted, however, that these results have been obtained always in tests at relatively high cyanide concentrations, at which most of the test animals even of the most resistant species and tested at the lowest temperatures were overcome within 6 hours. The fish tested at the highest temperatures turned over or died within about an hour or much sooner. In experiments with trout, the fish were overcome in a few minutes at high temperatures and within less than 20 minutes, on the average, at the lowest temperatures tested. Very different results have been obtained by British investigators in experiments with juvenile rainbow trout when these fish were exposed to relatively low cyanide concentrations in the Water Pollution Research Laboratory. Apparently, only very brief and incomplete reports of these interesting and important results have, as yet, been published, as items included in annual reports of progress of the Laboratory's work.

In one series of tests (Great Britain, Department of Scientific and Industrial Research, 1953), the young rainbow trout were exposed to different cyanide concentrations ranging from 0.125 to 1.0 mg/l as CN at each of three temperatures ranging from 12 to 22° C. At concentrations from 1.0 to 0.3 mg/l as CN, the expected relation between temperature and mean duration of survival, which was about 7 minutes or less, was again seen. The fish succumbed most rapidly at the highest temperature, and the relation between temperature and log survival time at each concentration, especially the two highest ones, deviated little from the expected linearity, or inverse proportionality. However, at concentrations from 0.25 to 0.175 mg/l, the temperature differences had no appreciable effect on the mean survival periods, which were all less than 18 minutes, and at the still lower concentrations of 0.15 and 0.125

mg/l, the trout tended to survive longest at the highest temperature and to succumb soonest at the lowest temperature. At the 0.15 mg/l concentration, the observed relation between log survival time and temperature was very close to direct proportionality; the slope of the straight line that could be fitted to the data was even greater than the slope in the opposite direction of either straight line that could be fitted to data obtained at the two highest concentrations tested. Even at the lowest tested concentration, the survival time of the trout usually was less than 200 minutes, so that not only at very slowly lethal cyanide levels was the unexpected relation to temperature demonstrable. It was noted that the deviation from the expected relation first became apparent at about the same concentration as that at which the relation between the logarithms of both cyanide concentration and mean duration of survival began apparently to deviate from linearity as the concentration was increased.

In the experiment just reported, no attempt was made, of course, to estimate and compare lethal threshold concentrations of cyanide at the different temperatures. The threshold values, however, are far more meaningful, of course, from a practical standpoint, than average periods of survival at rapidly lethal concentrations or lethal concentrations pertaining to arbitrarily selected, short exposure periods. In a much later experiment (Great Britain, Ministry of Technology, 1968), juvenile rainbow trout 3-5 cm long and acclimated to the test temperatures for 3 to 4 days were exposed for relatively long periods in enclosed vessels to different, continuously renewed cyanide solutions at temperatures of 12-13° C and 2-4° C. At the higher temperature, a not unusual, simple relation was observed between cyanide concentration and median periods of survival of the fish. A concentration tolerated by 50 percent of the fish for about 4 days apparently was near the lethal threshold level, which appears to have been in the neighborhood of 0.08-0.09 mg/l as HCN. The curve relating cyanide concentration to median survival periods at the low temperature, on the other hand, is highly irregular, with two sharp inflections at the survival time level of about 33 hours. Median survival periods in the neighborhood of 33 hours were recorded at different cyanide concentrations ranging from about 0.03 to about 0.06 mg/l as HCN, but the survival time increased to about 4 days at a cyanide concentration between 0.02 and 0.025 mg/l as HCN, which appears to have been approximately the lethal threshold level. Such an irregular relation between concentration of a toxicant and survival time is indicative of chronic toxicity which is physiologically (i.e., with respect to mode of action) quite distinct from rapidly lethal, acute toxicity. The toxic action of a single substance can be very different at high, rapidly lethal, and low, slowly lethal concentrations, and in a mixture of toxicants, different ones can be predominantly effective at different concentrations of the mixture. Each of two modes of action of a toxicant, or of two toxicants in a mixture, may be characterized by a very distinct relation between concentration and response or resistance time, and an irregularity such as that in question thus is apt to mark the transition from one mode of action to another. It should be noted that the indicated lethal threshold concentration of cyanide (0.02-0.025 mg/l as HCN) at the low test temperature (2-4° C) is much less than one-third of the corresponding value (0.08-0.09 mg/l as HCN) pertaining to the higher test temperature of 12-13° C. On the other hand, extension of that portion of the curve relating cyanide concentration to survival time at the low temperature which was fitted to data obtained at concentrations not less than 0.06 mg/l as HCN, so as to estimate the lethal threshold level by extrapolation from these data, would have indicated a threshold concentration of about 0.055 mg/l as HCN. This value is much more than one-half the corresponding value pertaining to the higher test temperature, which could be arrived at without such extensive extrapolation. Extension of the two curves in the opposite direction indicates that they would have met and crossed each other at a point corresponding to an HCN concentration of about

0.4 mg/l and a median survival time of about 100 minutes, if high enough concentrations had been tested to define the curves to the point of their intersection, which can be only very imprecisely estimated.

In two short paragraphs, without an accompanying graph, published one year later than the report just discussed, results of a further investigation very similar to the last-mentioned one have been reported (Great Britain, Ministry of Technology, 1969). Rainbow trout 2.5-2.6 cm long and acclimated for 3 to 4 days to the different test temperatures, which ranged from 4 to 20° C, were used. The cyanide solutions, tested in totally enclosed vessels, were prepared with a hard water, had pH values of 8.0 to 8.3, and were continuously renewed. It was reported that, at high cyanide concentrations, the fish died soonest at high temperatures, but at low concentrations at which the curves relating survival time to concentration of poison became almost parallel to the time axis, the fish died soonest at low temperatures. Unfortunately, the concentrations at which the different relationships of survival time to temperature were observed or at which the curves in question intersected were not reported. It was stated that the 72-hour median lethal concentration or tolerance limit, which is close to the median threshold concentration for survival, increased almost threefold with increase of temperature from 4 to 20° C, but the median lethal concentrations determined at the different temperatures at which tests were performed were not reported. Curiously, nothing at all was said again about the interesting irregularity of the relation between cyanide concentration and the median duration of survival at a very low temperature that had been previously reported. It is not clear, therefore, whether this irregularity was again observed or was found to have been probably attributable to error or some defect of the earlier experiment. The indicated difference between the threshold or nearly threshold levels of free cyanide at temperatures of 4 and 20° C is smaller than the difference between the corresponding values for temperatures near 3 and 12.5° C determined previously. This fact suggests that the threshold concentration at the low temperature indicated by the initial experiment perhaps was later found to have been erroneous (too low) for some reason, but one can only speculate about such matters in the absence of more complete information.

In a still later report of a related study at the same laboratory (Great Britain, Department of the Environment, 1972), some data on the influence of acclimation of young rainbow trout to a low temperature (3° C) on their resistance to cyanide poisoning at that temperature are presented. The fish were transferred from a temperature of 10° C to the test temperature of 3° C, and after varying periods of acclimation to the latter temperature ranging from nil to 7 days, their median lethal concentrations for uniform exposure periods of nearly 6, 12, and 24 hours were determined. When the periods of exposure to cyanide were relatively short (12 hours or less), the fish that had not been acclimated to the low test temperature proved most resistant to the poison, those that had been acclimated for only 1 day were next in resistance but much less resistant, and those acclimated for longer periods were least resistant, but there was no consistent difference in resistance among the groups acclimated for different periods greater than 1 day. When the duration of exposure to the cyanide solutions was 24 hours, no relation between resistance to cyanide (median lethal concentrations) and the duration of previous thermal acclimation was evident. The estimated 24-hour median lethal concentrations or tolerance limits for the different groups of fish were said to have averaged about 0.05 mg/l as HCN, but according to an accompanying graph, the average was nearer 0.04 mg/l, the individual values ranging from about 0.03 to about 0.055 mg/l as HCN. These results of toxicity tests of fairly short duration again reveal great sensitivity of the rainbow trout to free cyanide at very low temperatures, suggesting that the lethal threshold level at

3° C is less than 0.03 mg/l. The observed decrease of resistance to cyanide that results from acclimation to the low temperature evidently proceeds while the fish are exposed to eventually lethal levels and is so rapid that it is virtually complete before the end of a 24-hour exposure period.

Herbert and Merkens (1952) reported earlier that the mean duration of survival in KCN solutions with a cyanide concentration of 0.15 mg/l as CN and temperature of 17.5° C of rainbow trout previously held at unspecified, lower temperatures increased for some time as the fish became acclimated to the warmer water. The mean exposure periods to overturning of the fish were about 29 minutes after 1 and 2 days of acclimation to 17.5° C, 35 minutes after 4 days, 47 minutes after 5 days, 40 minutes after 172 hours (more than 7 days), and 51 minutes after 8 days, the longest acclimation period tried. The fish were exposed to cyanide more than once, but this did not appear to be the reason for their increased resistance.

It has been known for a long time that the resistance of fish to cyanide poisoning at a given temperature can change markedly as the animals become acclimated to that temperature after a large decrease or increase of the water temperature. After a few preliminary experiments with the marine longjaw goby, Gillichthys mirabilis, reported by Sumner and Wells (1935) had shown that such changes of resistance are demonstrable, the phenonenon was investigated in detail by Sumner and Doudoroff (1938), who used the same marine fish as their test animal. Gobies were acclimated for long periods to temperatures of 10, 20, and 30° C and then transferred for varying periods to temperatures higher or lower by 10° than the original acclimation temperatures. The mean duration of their survival in a M/1000 solution of NaCN (26 mg/l as CN) in sea water then was determined after each of a number of different periods of exposure to the new acclimation temperatures. In this way, it was possible to demonstrate conclusively that, at any given temperature, the fish were most resistant to the cyanide poisoning soon after their transfer to that temperature from a higher acclimation temperature and least resistant after transfer from a lower acclimation temperature. The effects of previous acclimation to higher or lower temperatures persisted for 3 days at least, and small remnants of them probably much longer. When some fish initially conditioned to 20° C were exposed for varying periods to the lower temperature (10° C) and others to the higher temperature (30° C), and all were then returned to the intermediate temperature for varying periods before exposure to cyanide at that temperature, the groups of fish previously held at the low and high temperatures differed markedly at first in their resistance. The persistence of this effect of previous acclimation to different temperatures on relative susceptibility to the poison at 20° C was found to increase as the duration of the previous exposure to the lower and higher temperatures increased. Thus, after acclimation to the low and high temperatures for only a day, the effect on resistance at the intermediate temperature persisted for a day but not for 3 days. When the acclimation to low and high temperatures lasted for 5 days, however, a significant difference between the mean survival periods of the groups of fish that had been held at these different temperatures was still demonstrable 3 days after return of the fish to the intermediate temperature.

Large differences in resistance to cyanide (M/1000 KCN solutions) of different populations of fishes of the genus Cyprinodon, and also of the poeciliid genus Crenichthys, inhabiting cool and warm springs in the Amargosa Desert of southwestern Nevada were observed by Sumner and Sargent (1940) when comparative tests were made at widely different temperatures to which these populations were accustomed. Although presumably isolated for thousands of years and thoroughly adapted to their warm-water environment, the populations inhabitating the warm springs were much less resistant at the high temperatures of these springs (32.5-33 and 35.5-37° C) than were populations inhabiting cool springs at the lower temperatures at which they were found (24 and 21° C). When tested at the same (common) temperature, low or high, the populations of the warm springs sometimes, but not always, proved more resistant to cyanide than were the populations of the cool springs.

It has been noted already that the 96-hour median tolerance limits of KCN for the bluegill at the temperature of 30° C (0.13-0.14 mg/l as CN) that have been estimated by Cairns and Scheier (1963) are considerably lower than the corresponding values (0.17-0.18 mg/l as CN) for the temperature of 18° C determined by the same authors in the course of the same, comparative study. These results were obtained in two bioassays, with soft and hard water, at each temperature. The 96-hour median tolerance limits, evaluated by static bioassays without renewal of test solutions, cannot be regarded as true lethal threshold concentrations, mainly because there was a fairly rapid loss of cyanide from the test solutions during the tests. Probably it was mainly for the same reason that the values obtained at 18° C are somewhat higher than the 72-hour median tolerance limit (0.154 mg/l as CN) determined at the temperature of 20° C by Doudoroff, Leduc, and Schneider (1966), who employed the constant-flow technique. It should be noted, however, that the latter tolerance limit is somewhat higher than the 96-hour median tolerance limits (also the 72-hour values, which were identical with the 96-hour values) at a temperature of 30° C estimated by Cairns and Scheier. It can be concluded, therefore, that the bluegill's long-term tolerance probably is indeed considerably greater at 18° C than it is at the much higher temperature of 30° C. This, of course, is the relationship that one would expect on the basis of the results of short-term tests. Unfortunately, no tests have been performed with the bluegill at very low temperatures such as 3 or 4° C, or even at 10° C. Perhaps its lethal threshold level of cyanide is maximal at a temperature that is neither high nor low, such as 15 or 20° C.

Hubault (1955a, 1955b) apparently found that the logarithms of the lowest cyanide concentrations at which cyprinids, Rutilus rutilus or Scardinius erythrophthalmus, are noticeably affected within 255-270 minutes decrease with rising temperature most markedly when the temperatures are extreme, high or low. The decrease of the logarithm of the effective concentration per degree of temperature increase seemingly was found to be much less over a wide range of intermediate temperatures, so that the relationship was representable by a decidedly sigmoid curve. However, Hubault's graphical presentation of his findings is very confusing. I was unable to determine the meaning of some unexplained lines and notations (numbers, arrows, etc.) on his graphs (Figure 1 in each paper), or even to determine with certainty which one of the many curves ("isochrones") shown there pertains to cyanide. Data to which the curves were fitted are not reported or plotted on the graphs. Observations apparently were made on small numbers of fish. In my opinion, the indicated relation between temperature and effective concentration of a toxicant is improbable.

Evidently, much still remains to be learned about the influence of temperature on the resistance of fish to cyanide. The relatively low resistance of rainbow trout to low, slowly lethal concentrations of free cyanide at low temperatures that has been reported probably is a result of decrease of the rate of detoxification at reduced temperatures. When cyanide concentrations are high, the detoxification mechanism must be soon overwhelmed by the rapid influx of the poison, and the rate of mortification must become dependent only on the rates of other, continuing, vital processes that constitute the disorganized, general tissue metabolism. The speed of death then can be seen to be directly related to the metabolic rate. Inasmuch as the fatal tissue concentration level of a poison may decrease with increase of the metabolic rate, and also because the rate of entry of the poison can increase with rise of temperature (because of accelerated external respiration, etc.), an increase of the detoxification rate at an elevated temperature need not necessarily result in a higher lethal threshold concentration in the external medium. If, however, at a high temperature, but not at a low temperature, a poison is eliminated from the body as rapidly as it enters before a lethal internal concentration is reached, the animal can continue to withstand at the high temperatures an external concentration that is intolerable, causing death eventually, at the low temperature. When the cyanide dose is massive, sluggish and hardy warm-water fishes, such as the longjaw goby, *Gillichthys mirabilis*, can live very much longer at a low temperature than can active and sensitive cold-water fishes, such as trout, exposed to high concentrations of the poison at the same temperature. But it is reasonable to suppose that neither the rainbow trout nor the family Salmonidae is unique in having lower lethal threshold concentrations of cyanide at low temperatures than at higher temperatures. Results similar to those obtained in the experiments with rainbow trout were said to have been obtained also in tests with the roach, *Rutilus rutilus*, (Great Britain, Ministry of Technology, 1968), but supporting data were not presented. Alabaster *et al*. (1972) stated that juvenile roach proved much more sensitive to cyanide than rainbow trout in 48-hour tests at 3-4° C. The published information on the relation to temperature of lethal threshold levels of cyanide even for rainbow trout is incomplete, and such information pertaining to other fishes is certainly quite inadequate. The transient influence of recent thermal experience (acclimation) of fish on their resistance to high concentrations of cyanide at any given temperature is interesting but probably not of major practical importance.

INFLUENCE OF DISSOLVED OXYGEN

Pronounced increases of the susceptibility of fish to cyanide at reduced concentrations of dissolved oxygen have been observed repeatedly in both static toxicity tests (Southgate, Pentelow, and Bassindale, 1933; Wuhrmann and Woker, 1953, 1955) and experiments with continuously renewed solutions (Downing, 1954; Burdick, Dean, and Harris, 1958). Downing (1954) found the influence of dissolved oxygen on the survival time of yearling rainbow trout (at 17° C and pH 7.8-8.2) to be greatest at the lowest cyanide concentration tested (0.105 mg/l). At this concentration, there was a hundredfold increase of the median survival time (time to immobilization) with increase of dissolved oxygen from about 3 to about 9 mg/l. At the higher cyanide level of 0.116 mg/l, the corresponding increase was not much more than a tenfold increase, and at 0.155 mg/l, a level at which the median immobilization time was less than 17 minutes even at a high oxygen concentration, the effect was much smaller. Wuhrmann and Woker (1953, 1955), in experiments with the minnow *Phoxinus laevis* at three different temperatures, also found the effect of dissolved oxygen on resistance to cyanide (overturning time) to be very pronounced at low levels of cyanide but not at high levels. In Downing's (1954) experiments, the effect of a 1 mg/l dissolved oxygen increment on resistance to cyanide was not found to decrease as the air-saturation value was approached. At low cyanide concentrations, the effect (proportional increase of the immobilization time) was most pronounced at the highest dissolved oxygen levels. However, the data of Wuhrmann and Woker (1953, 1955), and also those of Southgate, Pentelow, and Bassindale (1933), who used rainbow trout as test subjects as Downing did, but at a temperature of 7-9° C, indicate a more pronounced effect at low dissolved oxygen levels than at high levels.

Only Burdick, Dean, and Harris (1958) are known to have estimated lethal threshold concentrations of cyanide at different, constant levels of dissolved oxygen for comparative purposes. As noted already, they estimated that reduction of the oxygen concentration (by means of nitrogen) from a level near the air-saturation level to 5.2 mg/l caused a reduction of the lethal threshold concentration of cyanide for brown trout by only about 0.01 mg/l (from 0.09 to 0.08 mg/l as CN) at a temperature of about 15.5° C. Reduction of the oxygen concentration to

4.2 mg/l was estimated to have caused reduction of the lethal threshold concentration of cyanide for smallmouth bass, Micropterus dolomieui, by less than 0.02 mg/l or about 17 percent (from 0.104 to 0.086 mg/l as CN) at 21° C. The mean periods of survival (to overturning) of the trout or the bass at the high and reduced oxygen concentrations are strikingly different (i.e. shorter at the low dissolved oxygen levels) when the cyanide concentrations are equal and low. But they differ relatively little when the cyanide levels are equal and high, and the differences of the estimated lethal threshold levels of cyanide at the different oxygen concentrations are not very impressive. Apparently, when dissolved oxygen concentrations do not fall below 4 mg/l or much below 50 percent of air-saturation levels, the influence of their wide variations on the maximum cyanide concentrations tolerated indefinitely by fish is not of great importance. In the experiments of Burdick, Dean, and Harris, the individual survival (overturning) times of the fish at low cyanide and low oxygen concentrations were extremely variable. Also, the maximum geometric mean resistance periods recorded in the tests at reduced oxygen concentrations were rather short (202 and 362 minutes for the trout and the bass, respectively), though they were much longer than the maximum mean or median values recorded by the other investigators whose comparable results have been summarized above. The indicated lethal threshold values derived by extrapolation from the data in question are not likely to be very accurate estimates, but they probably are not seriously misleading.

Cairns and Scheier (1958) found that the 96-hour median tolerance limit of KCN for bluegills at 18° C was reduced to about 0.05 mg/l as CN by daily reduction of the dissolved oxygen content of the standing test solutions (by means of nitrogen) to about 2 mg/l for a period of about 2 hours. Gradual reduction of the oxygen concentration to the low level from the air-saturation level and its subsequent return to the high level by aeration each was accomplished in about 3 hours. In the same study, the 96-hour median tolerance limit for controls held at "normal" (relatively high) oxygen concentrations of 5 to 9 mg/l was found to be 0.18 mg/l as CN. It appears that the occurrence of very low but not lethal concentrations of dissolved oxygen even for limited periods can seriously depress the cyanide tolerance of fishes.

INFLUENCE OF WATER SALINITY AND HARDNESS

Broderius (1973) found that threespine sticklebacks exposed to NaCN solutions of equal cyanide content died much sooner in solutions prepared with sea water having a chlorinity of about 16-17 parts per thousand than in solutions prepared with half-strength, diluted sea water or with fresh water. The pH of each test solution averaged about 7.7. At a cyanide concentration of 0.27 mg/l as CN in fresh water, in the 50 percent diluted sea water, and in sea water, the median survival periods (at 20° C were 412, 371, and 198 minutes, respectively; at a concentration of 0.21 mg/l as CN, they were 642, 582, and 350 minutes, respectively. It can be seen that in the medium that was half sea water and half fresh water, the cyanide was only a little more toxic than it was in fresh water, so that the much greater toxicity in the undiluted sea water could hardly have been due to any chemical interaction between the cyanide and the sea salts. And there was no consistent relation between the salinity of the solutions and their molecular HCN content. The determined molecular HCN concentrations in the test solutions containing 0.21 and 0.27 mg/l of cyanide as CN (amounts added to the water, averaging 0.24 mg/l) that were prepared with sea water, 50 percent sea water, and fresh water averaged 0.206, 0.203, and 0.206 mg/l, respectively. These data indicate some loss of cyanide from all the solutions but only fortuitous variation of the individual, determined levels of molecular HCN in waters of varying salinity to which the same amount of NaCN had been added. Both the sticklebacks and the sea water had been obtained from an estuarial source. The salinity of this sea water, in the neighborhood of 30 parts per thousand, was well below that of full-strength, oceanic water (about 35 parts per thousand), and so it could not have been an unduly saline, unfavorable medium for the euryhaline sticklebacks, which commonly inhabit such water. The sticklebacks, collected in salt water of unknown salinity, had been acclimated for about a week before the tests to different waters with the salinities of the test solutions to which they were subsequently exposed.

According to an earlier report (Great Britain, Ministry of Technology, 1968), results of experiments with juvenile rainbow trout 4-5 cm long, at temperatures of 11-22° C, had suggested that the toxicity of HCN is not related to water salinity. However, the data on which the tentative conclusion was based were not presented. The most saline water tested was 70 percent sea water. The trout had been acclimated to the different water salinities. Salinity of the water may not influence the susceptibility to free cyanide of all euryhaline fishes in the same way.

There is some published evidence that the hardness or the total alkalinity of water per se can have a material effect on the toxicity of free cyanide to fish, but it is not convincing; contradictory findings pertaining to this matter have been reported. When sufficiently high pH values are involved, pH differences associated with differences of carbonate hardness or total alkalinity can, of course, be reasonably expected to have some effect. Henderson, Pickering, and Lemke (1961) did report a considerable difference of 96-hour median tolerance limits of NaCN for fathead minnows in a soft water and a hard water at 25° C; these estimated limits for the soft and hard waters, evaluated by static bioassays without renewal of test solutions, were 0.23 and 0.35 mg/1 as CN, respectively. The alkalinity and hardness of the soft water (pH 7.4) were 16 and 20 mg/1 (as $CaCO_3$), respectively, and those of the hard water (pH 8.2) were 320 and 380 mg/1, respectively. The pH of the well buffered, hard water (8.2) presumably did not increase in the presence of respiring test animals, and such a pH is not high enough to have greatly depressed the toxicity of the free cyanide by increasing the ionization of HCN. Therefore, the observed difference in toxicity of NaCN solutions prepared with the two waters appears to be attributable to the difference in hardness or of total alkalinity. However, the difference of results obtained with the two waters may have been fortuitous. No difference of the toxicity to fathead minnows in the two waters of acetaldehyde cyanohydrin (lactonitrile), whose rapid hydrolysis in aqueous solutions yields free cyanide, was observed by the same investigators.

Cairns and Scheier (1963) observed no difference of the toxicity of KCN to bluegills in their synthetic soft and hard waters. They estimated the 96-hour median tolerance limits of KCN in each water at two different temperatures, and the means of their values for the two waters are virtually identical. However, the difference between these waters was not great; the alkalinity and hardness of the soft water were a little more than twice the corresponding values for the soft water used by Henderson, Pickering, and Lemke, and those of the hard water were a little less than half the corresponding values for the hard water of the latter authors.

Leclerc and Devlaminck (1950) found KCN to be some two to three times as toxic to minnows, Phoxinus laevis, in a hard, natural water than in distilled water at 20° C. But the duration of their static bioassays whereby their minimum lethal levels of the poison were determined was only 6 hours, and the pH values of the test solutions were not reported. Perhaps the hydrolysis of KCN added to the unbuffered, distilled water caused sufficient elevation of the initial pH of the solutions to depress the toxicity of the free cyanide markedly for all or most of the duration of the very brief tests.

Burdick, Dean, and Harris (1958) found no difference of the toxicity of KCN to brown trout in two natural waters, an acid stream water with a total alkalinity of 6 to 12 mg/l as $CaCO_3$ and a moderately hard spring water with a total alkalinity of about 100 mg/l and pH 7.5-8.3. Mean survival periods at various concentrations of cyanide (0.145 to 2.14 mg/l as CN) in the two waters were determined and found to fit the same curve (relating log time to log concentration) when plotted against the cyanide concentration. Most of the mean survival periods recorded were within the range of 5 to 100 minutes.

EFFECTS OF ACCLIMATION TO CYANIDE

The influence of acclimation of fish to low, sublethal levels of cyanide upon their subsequent resistance to the poison has been investigated, but not thoroughly. Malacea (1968) found that the median survival periods of minnows, *Phoxinus phoxinus*, at a cyanide (KCN) concentration of 0.5 mg/l as CN were increased considerably, by about 60 to 70 percent, by previous exposure of the fish for 2 days to a lower cyanide level of 0.1 mg/l as CN. Neil (1957), however, reported varying results of experiments with brook trout, *Salvelinus fontinalis*, which were acclimated for 15 days or longer to one of three sublethal concentrations of KCN (0.01, 0.03, and 0.05 mg/l as CN) and then exposed to different lethal levels. The acclimated trout proved sometimes more resistant and sometimes less resistant than the unacclimated controls, the effect of acclimation varying with the acclimation level of cyanide and with the lethal level tested. Fish acclimated to 0.01 mg/l were consistently the most resistant ones, on the average. Those previously exposed to the higher sublethal levels (i.e., to 0.03 and 0.05 mg/l as CN) were less resistant; they proved less resistant even than the controls to 0.30 mg/l, but more resistant than the controls to 0.40 and 0.50 mg/l, the resistance time varying inversely with the acclimation level. It appears that previous exposure to some low, sublethal levels of cyanide can increase somewhat the resistance of the trout to lethal levels by setting in motion some adaptive mechanism, but the adaptation can be largely or entirely canceled or counterbalanced by concomitant injury when the fish are exposed to higher sublethal levels. That injury evidently can result in reduced resistance to lethal cyanide levels.

Brockway (1963) found that groups of cichlids, Cichlasoma bimaculatum, about 4 months old and 1 g in average weight, that had been acclimated for 40-42 days to cyanide (NaCN) concentrations of 0.02, 0.06, and 0.10 mg/l as CN were all more resistant than unacclimated controls to not very rapidly lethal cyanide levels. Their 24-hour median tolerance limits at 25° C were estimated to have been 0.22, 0.24, and 0.24 mg/l as CN, respectively, whereas the corresponding value for the controls was 0.18 mg/l. However, when a group of fish of the same age and size acclimated for 60-62 days to a cyanide concentration of 0.10 mg/l as CN were exposed to the relatively high, rapidly lethal cyanide level of 0.42 mg/l, the acclimated fish proved far less resistant than the unacclimated controls. The determined median periods of survival were 340 minutes for the controls but only 140 minutes for the fish previously exposed to cyanide. The significance of these results is unclear. They are not in agreement with Neil's, whose brook trout acclimated to relatively high but tolerable cyanide levels proved less resistant than controls to relatively low lethal levels but more resistant than the controls to higher lethal levels. They also do not agree with Malacea's observations on minnows. When all of the available data on the effects of acclimation to cyanide on lethal levels are considered together, they are quite puzzling and indicate a need for further investigation of this matter. All of the lethal levels of free cyanide to which fish were exposed in the reported experiments were more or less rapidly lethal and well above the lethal threshold concentrations. What needs to be determined is whether the lethal threshold concentrations are or are not increased materially by acclimation of fish to somewhat lower levels.

RESISTANCE IN RELATION TO BODY SIZE AND PHYSIOLOGICAL STATE

There is no uniformity of the results of investigations also of the relation between the size of fish of a given species and their resistance to cyanide poisoning. The resistance of different species has been reported to decrease, increase, or not change appreciably with large increases of body size. Species of fish may indeed be unlike in this regard, but other factors such as difference of experiments in the manner of evaluation of relative resistance or in relationship between the size of fish and their age may have been largely responsible for the lack of agreement of reported experimental results.

Herbert and Merkens (1952) compared the mean periods of survival of groups of yearling rainbow trout (i.e., fish believed to be of nearly the same age) differing widely in mean body length in a solution of KCN whose concentration (about 0.153 mg/l as CN) proved quite rapidly lethal to all of the fish, killing most of them within 40 minutes. The individual lengths of the fish ranged from 5.5 to 17.25 cm. The larger fish tended to succumb much sooner than the smaller ones. For example, the mean periods of survival of thirteen fish averaging 15.0 cm in length, ten fish averaging 9.0 cm in length, and eleven fish averaging 7.1 cm in length were 18.4, 24.1, and 37.0 minutes, respectively. Herbert and Downing (1955) noted a statistically significant negative correlation between body length and overturning time of rainbow trout in KCN solutions when the fish were exposed to cyanide concentrations of 0.125 and 0.150 mg/l as CN (levels apparently then lethal to the average fish within 1 hour or much sooner), but not when they were exposed to a higher concentration, 0.20 mg/l as CN. There is no apparent reason to question the reliability of the above findings, but it would be interesting to know how, or to what extent, the differences in resistance to cyanide may have been related to other physiological differences that had resulted in large differences in size of fish of the same age. Would fish of different age but of average size for their age have differed correspondingly in their resistance, having become increasingly susceptible as they grew larger? It has seemed reasonable to suppose that the resistance of fish to acute cyanide poisoning, which is believed to interfere with internal or tissue respiration, generally tends to increase with decrease of metabolic rate (Sumner and Doudoroff, 1938). And the metabolic rate of a juvenile fish is not likely to increase as the fish grows larger; it tends to decrease, as a general rule. However, it should be recalled that the resistance of rainbow trout to not very rapidly lethal cyanide concentrations has been reported to decrease with reduction of temperature, which certainly depresses the rate of respiratory metabolism.

Anderson (1974), in experiments in which only mature, male guppies were used, found a definite, direct relation between body weight and resistance to KCN. A linear regression with a high correlation coefficient was defined by an appropriate equation. The fish were segregated for the tests in groups of ten, and exposed for 96 hours to continuously renewed KCN solutions at a temperature of 25° C. The 96-hour median lethal concentration in mg/l for fish of a given size class was shown to be equal to 0.147 times $W^{0.72}$, where W is the weight in grams of a group of ten fish of that size class (or the weight of an individual fish of average size multiplied by 10). Although the value to be derived by the use of this equation was said to be the median lethal concentration of "potassium cyanide", I am convinced, for several reasons, that this is an error and that the concentration in question actually is, or was meant to be, the median lethal concentration of cyanide as CN. As noted already, other investigators have found the guppy to be highly resistant to cyanide poisoning, their data indicating 96-hour median tolerance limits of free cyanide much above 0.15 mg/l as CN. Thus, even 0.147 mg/l as CN is a surprisingly low value for the 96-hour tolerance limit for the 0.1 g, mature males, and 0.147 mg/l as KCN, or 0.059 mg/l as CN, hardly could be a correct value. Free cyanide as CN doubtless was measured when the test solutions were analyzed.

Cairns and Scheier (1959) determined by static bioassays at 20° C the 96-hour median tolerance limits of KCN for three groups of bluegills averaging about 3.9, 6.1, and 14.2 cm in length and about 1.0, 2.8, and 54 g, respectively, in weight. These fish doubtless were not all of nearly the same age. The reported tolerance limits for the groups of fish of small, medium, and large size were 0.55, 0.45, and 0.57 mg/l as KCN (about 0.23, 0.19, and 0.24 mg/l as CN), respectively, and it was concluded that there was no significant difference in resistance to KCN of the bluegills of different sizes. The experimental material was obtained from two different sources and it is not clear whether or not this could have materially affected the result of the experiment, since the relative numbers of fish from the two sources and their size distributions were not reported. Also, although the test solutions were said to have been analyzed for cyanide to "make certain that a rather constant concentration of cyanide ions was maintained throughout the experiment," the results of these analyses and of determinations made of dissolved oxygen were not reported. It is unlikely that the cyanide concentrations in the gently aerated test solutions remained quite constant for 96 hours and that dissolved oxygen concentrations in all test vessels (each containing ten small, ten medium, or five large bluegills) were identical. Since the fish of different sizes were not exposed together to the same solutions, even moderate differences among initially like solutions to which they were exposed could have materially influenced the test results, perhaps concealing considerable differences in cyanide tolerance of fish differing greatly in size.

Sumner and Doudoroff (1948) found no significant difference in resistance to a very high concentration of KCN (M/1000) between small longjaw gobies, *Gillichthys mirabilis*, and large ones whose average weight was four to five times that of the small ones. Wells (1916), however, has presented data showing that the average survival periods of rock bass, *Ambloplites rupestris*, exposed to a concentration of KCN (M/25,000, or

1.0 mg/l as CN) that was also quite rapidly fatal increased markedly with large increases of body size and, doubtless, of the age of the fish. In his experiment on effects of starvation on cyanide tolerance, survival periods of unstarved controls weighing 1-1.5, 10-15, 25-40, and 80-200 g averaged 71, 106, 141, and 166 minutes, respectively. The corresponding values for his experimental fish starved for varying periods are 87, 107, 142, and 170 minutes, respectively. There was no consistent difference in resistance shown between the smaller and larger fish in the 80 to 200 g weight range, the average survival periods of fish weighing 130-200 g having been not much greater than those of fish weighing 80-95 g, and those of fish weighing 100-125 g having been much less. The number of fish tested was small. Variations of the resistance of fish to cyanide with body size thus may be often, but not always, readily demonstrable, and the resistance apparently can either increase or decrease with increase in size.

Wells (1916) believed that he was able to demonstrate effects of starvation on the resistance of rock bass to cyanide, which he thought initially increased and then decreased with increasing duration of starvation. Although there may be such effects, my own analysis of Wells' data led me to the conclusion that no effect actually has been demonstrated by the few experiments performed by him. After 47 days of starvation, the rock bass did survive longer in a KCN solution (1.0 mg/l as CN) than controls did in five of six trials with fish of different size, but this result could have been fortuitous. And in the other tests, with fish starved for 12 and 52 days, no effect or consistent difference between the experimental and control fish was apparent to me.

Costa (1966) found that minnows, Phoxinus phoxinus, that had been exercised before their exposure to NaCN solutions lost their equilibrium in very rapidly lethal cyanide solutions sooner than did unexercised controls. At lower cyanide concentrations that were still highly toxic, causing loss of equilibrium after about 1 hour of exposure, there was little or no difference, however, between the responses of the exercised fish and the controls.

Pronounced differences in resistance to cyanide between lots of fish of the same species and nearly the same size but obtained from different sources and/or tested at different times have been recorded. The reasons for these differences are unknown. Such differences in resistance of young rainbow or brown trout, due perhaps to inherent physiological differences between the stocks, to seasonal variations in physiological state, or to differences in thermal history, have been reported by British investigators (Great Britain, Department of Scientific and Industrial Research, 1953; Herbert and Downing, 1955) and by Burdick, Dean, and Harris (1958). Herbert and Merkens (1952) and Herbert and Downing (1955) found a strong correlation between the overturning times in separate trials of individual rainbow trout exposed to KCN solutions and returned to clean water for recovery to permit repetition of the relative resistance tests with the same fish. Differences in size of the fish and the observed relation between resistance to cyanide and body size must have been partly responsible for the above correlation. Effects of thermal acclimation, as well as of previous exposure to sublethal levels of cyanide, on the resistance of fish to the poison, already have been discussed. Heritable (genetic) and inherent seasonal differences of resistance have not been studied, but, as suggested above, may well be major reasons for variability of experimental results obtained by the most refined methods.

INTERACTIONS OF FREE CYANIDE WITH OTHER POISONS

The acute toxicity to fish of specially prepared mixtures of toxicants including free cyanide as one of the components has been studied by several investigators. The toxicity of various, individual metal-cyanide complexes will be fully considered in another section of this review (Section V). There, the interaction of free cyanide and individual heavy metal cations, such as zinc ion, which combine to form these complexes or derive from their dissociation, also will be discussed. Here, only interactions of cyanide with toxicants other than the heavy metals, or with metals present in mixtures together with other toxicants, will be considered.

Southgate (1932) reported that marked increases of the toxicity to rainbow trout of rather rapidly lethal solutions of p-cresol resulted from the addition to them of sublethal or not nearly as rapidly lethal amounts of KCN. No corresponding increases, except a small increase in one instance, of the toxicity of rapidly lethal KCN solutions (reported as the reciprocal of overturning time) resulted from similar additions of p-cresol in sublethal or much less rapidly lethal amounts. Thus, although some infra-additive, or less-than-additive, interaction (Warren, 1971; Sprague, 1970) of the two toxicants was revealed, their joint action certainly was not shown to be nearly additive. Because of the high (rapidly lethal) concentration of one or the other toxicant in each of the mixtures, all of which caused overturning of the fish of average resistance within 25 minutes or less, the practical significance of these observations is uncertain.

Pronounced synergism of free cyanide and ammonia (molecular base), observed in experiments with the chub (cyprinid) Squalius cephalus at 12-14° C, has been reported by Wuhrmann and Woker (1948). Again the tests were of rather short duration, but some tests of solutions with concentrations of both toxicants apparently near or below lethal threshold levels of the individual toxicants were included in the small series of trials of different combinations. Curiously, when free cyanide concentrations were very high (7.6-15.2 mg/l as CN) and not by themselves much more rapidly lethal than were much lower concentrations, the lethal action was accelerated in the presence of small, relatively harmless amounts of molecular ammonia (NH_3) much more markedly than it was at the lower levels of cyanide. Indeed, when the cyanide concentration was about 2.3 mg/l as CN, the presence also of molecular ammonia had no effect when its level was 0.28 mg/l and only a moderate effect when the level was 0.65 mg/l; yet, only 0.55 mg/l had a striking effect when the cyanide concentration was more than 15 mg/l, for the rate of intoxication then increased much more than threefold. Of more interest from a practical standpoint, however, were two tests in which cyanide concentrations of 0.10 and 0.14 mg/l as CN were combined with NH_3 concentrations of 0.70 and 0.35 mg/l, respectively, and the two mixtures found to be decidedly toxic, the fish having turned over in only 78 and 225 minutes, on the average, and died in 156 and 300 minutes, respectively. In the absence of the cyanide, an NH_3 concentration of 0.70 mg/l did not cause overturning of the fish within an observation period of 500 minutes or more, and 1.3 (1.2-1.5) mg/l caused overturning only after about 215 minutes, on the average. Cyanide alone did not cause overturning or death of *Squalius* within the observation period at a concentration of 0.20 mg/l as CN, and a concentration of 0.50 mg/l caused overturning only after 141 minutes and death after 268 minutes of exposure, on the average. It is obvious that the toxicity of the mixtures was far greater than that attributable to independent action of either one of the two components known to be highly toxic, but the design of the experiments was not such as to permit complete determination of the nature and degree of interaction.

Lloyd and Jordan (1963, 1964) compared the toxicities to rainbow trout (i.e., the median tolerance limits, or toxicity indices derived therefrom) of various sewage effluents containing cyanides with their "predicted" toxicities. The predicted toxicities were computed values based on chemical analyses of the effluents, available data on the individual toxicities of their known toxic components (tested separately), and the assumption that the toxicity of each of these mixtures of poisons is equal to the sum of the individual toxicities of the identified and measured toxic components. In other words, recognition and appropriate measurement of all the important toxic components of the effluents, and also strictly additive joint action of these components (also known as additive synergism) were assumed. Fair agreement of the observed toxicities of the effluents and those predicted by the summation of the toxicities of their known toxic constituents (expressed in toxicity units) was found in many but not all instances. Cyanide apparently was not one of the major or important constituents of some of the effluents, though present in measurable amounts. The authors realized that some of the cyanide and certain heavy metals, such as copper and nickel, could have been present in the effluents in the form of relatively harmless metal-cyanide complexes (complex anions) and that their measurements of "free cyanide" were not reliable. They recognized the possibility that disagreement of observed and predicted toxicities in some instances could well have been due to this source of error. Similar studies of heavily polluted river and estuarine waters have been reported by Brown, Shurben, and Shaw (1970). These authors, however, used a new and apparently fairly reliable method for determination of free, molecular HCN. They and Lloyd and Jordan have underestimated, sometimes rather seriously, the toxicities of many of the polluted waters or effluents examined; in other words, the observed toxicities often have been considerably greater than the predicted ones. Since the reasons for disagreement of the observed and predicted toxicities of such complex mixtures of incompletely known composition can never be all definitely established, studies like those just described cannot, of course, throw much light on the interactions of cyanide with other poisons in the mixtures. Many more experiments with various mixtures of known and adjustable composition obviously are needed.

It has been reported (Great Britain, Ministry of Technology, 1969) that, when cyanide and phenol at concentrations of equal toxicity to rainbow trout were combined in water with salinity less than 20 percent of the salinity of sea water, the toxicity of the mixture to the trout was near that which could be predicted on the basis of the assumption of strictly additive interaction of the two toxicants. When, however, the water salinities were between 50 and 70 percent of the salinity of sea water, the observed 48-hour median lethal concentration of the mixture was about 1.7 times the value so predicted.

Anderson (1974) evaluated the toxicity to mature, male guppies of mixtures of the cyanide and the pentachlorphenate of potassium, as well as of each of the individual toxicants, in continuously renewed solutions tested for 96-hour exposure periods. He found that the toxicity of the mixtures was less than that predictable on the basis of the assumption of what he termed "concentration additive" interaction of the toxicants (i.e., the kind of interaction assumed by Lloyd and Jordan and by Brown, Shurben, and Shaw), even though the concentration-response curves (regression lines relating percent mortality probits to log concentration, with appropriate correction for mean body weight differences) for the two individual toxicants proved similar in slope. So-called "response addition", or "independent joint action" of the two toxicants, rather than "concentration addition" ("toxic unit summation" or "similar joint action" of the poisons), was suggested by Anderson's data but was not definitely established. For an explanation of these terms, adopted by Anderson, and of related, interesting concepts, the reader must be referred to Anderson's original work. Only a very brief explanation of the terms can be given here.

When two quite independently acting toxicants occur together, each in concentration sufficient to kill some (e.g., 50%) but not all fish in a given exposure period, some of a group of fish exposed to the mixture that would not be killed in that period by one of the toxicants may be killed by the other. The result is one kind of "response addition". True "concentration addition" occurs when two toxicants in a mixture (the concentrations of both of which may be below their individual effective or lethal levels) act together in effecting a response, such as death, as though they were one and the same toxicant, except that their equally toxic concentrations may be quite different; their relative concentrations in a mixture to whose toxicity they contribute equally must be inversely proportional to their individual toxicities. I must point out here that I believe Anderson erred when he stated, in his footnote on page 54, that the equation given for derivation of the proportion of individuals responding to a mixture when there is only response addition involves the assumption of "a total positive correlation of tolerances by the test animals to each of the toxic constituents." On the contrary, an unlikely total lack of such correlation must be assumed, I believe, if the equation in question is to be accepted as a valid or appropriate representation of response addition. A true lack of any interaction or joint action of two toxicants, a hypothetical situation perhaps never fully realized but postulated by Warren (1971), for example, requires a total positive correlation of relative, individual susceptibilities of the test animals to the two toxicants and, therefore, the absence of any response addition.

Cairns and Scheier (1968) were unable to demonstrate any joint toxic action or synergism of KCN, naphthenic acids, and potassium dichromate, $K_2Cr_2O_7$ (the last present only in amounts far below lethal levels) when these toxicants were combined in solutions and the 96-hour median tolerance limits of the mixtures for bluegills determined. The result of one such bioassay of a solution in which the relative amount of cyanide was quite small, so that it could not have contributed much to the toxicity of the mixture, did suggest the possibility of an interaction of this nature. However, in another, similar test of a mixture in which the relative amount of cyanide was much greater, some possible antagonism of the combined toxicants, rather than any kind of synergism, was indicated. The reported 96-hour median tolerance limit of the mixture was a level at which the concentration of naphthenic acids alone was sufficient, and that of the cyanide (0.24 mg/l as CN) apparently much more than sufficient, to kill 50 percent of the test animals in 96 hours. The 96-hour tolerance limits of the individual toxicants and of the mixtures were determined for comparative purposes, at 18° C, by static bioassay with mild, controlled aeration and no renewal of test solutions, and these test results (estimates) cannot be deemed entirely reliable.

Brockway (1963) found that juvenile cichlids, Cichlasoma bimaculatum, that had been exposed for 60-62 days to a sublethal concentration of sodium pentachlorphenate (0.02 mg/l as pentachlorphenol) were less resistant than controls were to a high, lethal concentration of free cyanide (0.42 mg/l as CN), to which they were subsequently exposed. Their median survival time in the cyanide solution was 253 minutes, and that of controls was 340 minutes. Resistance to a lethal level of pentachlorphenate was not reduced, but cichlids that had been exposed for 60-62 days to a high but sublethal level of free cyanide (0.10 mg/l) proved slightly less resistant than controls were to the lethal concentration of sodium pentachlorphenate (1.1 mg/l as pentachlorphenol).

ANTAGONISTIC ACTION OF THIOSULFATE

Under certain conditions, fish have been reported to have been evidently protected against the lethal action of free cyanide by the presence in the cyanide solutions of the thiosulfate (hyposufite) ion, $S_2O_3^{-2}$, in relatively high concentrations (Achard and Binet, 1934; Costa, 1965) or by previous exposure of the fish (young carp, Cyprinus carpio) to solutions of this antidote (Achard and Binet, 1934). In experiments in which the concentration of sodium thiosulfate, $Na_2S_2O_3$, was constant (0.025N, or 1,975 mg/l), Costa (1965) found the antidote to be most effective at low cyanide concentrations, affording little or no protection against very rapidly lethal cyanide levels. None of the effects on survival time that he observed in experiments with several species of fish, such as a mere doubling of the survival time at a given cyanide concentration, are very impressive, but more striking positive results perhaps could have been obtained by performing tests at more slowly lethal levels of cyanide. Weiss, Abramson, and Baron (1958) reported that $Na_2S_2O_3$ did not block the lethal action of cyanide in the Siamese fighting fish, Betta splendens, under the particular conditions of their experiments.

The reported antagonistic or antidotal effect of thiosulfate is believed to be directly related to its role in the internal detoxification of cyanide, an enzyme-catalyzed process or reaction whereby the cyanide radical is combined in animal tissues with sulfur, the thiocyanate so produced being relatively harmless. This antidotal effect is of some physiological interest, but in view of the high thiosulfate concentrations in the external medium that are apparently required for effective protection of fish, it can hardly be reasonably regarded as having any practical significance or value in connection with waste disposal problems. The pertinent data will not, therefore, be considered here in detail.

FIELD OBSERVATIONS OF CYANIDE-CAUSED FISH MORTALITIES

Some observations made in the field on fish mortalities in waters polluted with cyanide have been in fair agreement with the results of toxicity tests performed in the laboratory. Grau and Hrubec (1965), for example, reported that a September fish mortality in a polluted river ended at a point downstream from the source of temporary pollution where the determined cyanide concentration had declined to 0.14 mg/l as CN. However, some of the cyanide measured may not have been in the free state. In reporting results of another, similar study, Moore and Kin (1968) stated that "analytical tests on the receiving stream and observations made on fish in the stream and in live boxes show that the free cyanide was lethal to fish at concentrations above 0.1 mg/l, was lethal to most game fish at concentrations of 0.05 to 0.1 mg/l, and some fish at concentrations as low as 0.03 mg/l." These field observations were made in winter, when water temperatures were near the freezing point. The low temperature may have been responsible for the reported, unusually high sensitivity of some of the warm-water fish to the free cyanide, which derived from hydrolytic decomposition of spilled acetone cyanohydrin, $(CH_3)_2C(OH)CN$. However, the data on which the conclusions of Moore and Kin were based were not reported in full detail, and it is not obvious that the observed fish mortality could be correctly attributed to the measured cyanide alone. There is no apparent reason to believe that serious analytical errors were involved, but several chemicals other than cyanide also were or may have been involved. Free chlorine was introduced intentionally into the stream water by addition of calcium hypochlorite to reduce the concentration of cyanide, converting it to cyanate, and chlorine is known first to combine with free cyanide so as to form very toxic cyanogen chloride, $CNCl$. The pollution incident investigated evidently was not a simple one. Indeed, the authors themselves stated that the "characteristic odor of cyanide in the stream water gave a clue to at least one cause of the kill" (emphasis added), thus suggesting that they were not sure that free cyanide was the only harmful agent present.

Bassindale, Southgate, and Pentelow (1933) reported some observations made in the field on the color of the gills of Atlantic salmon, *Salmo salar*, and anadromous brown trout ("sea trout") smolts that were still alive but dying in cyanide-polluted water of the estuary of the River Tees. They found the gill color of the dying fish, measured on a color scale by comparison with specially prepared color standards arranged in a graded color chart, was a decidedly brighter red color, on the average, than that of the gills of normal fish. A similar brightening of the red color of the gills was observed also in brown (sea) trout and rainbow trout exposed to lethal cyanide solutions in the laboratory, and it was attributed to interference by cyanide with tissue respiration and the consequent, abnormally high degree of oxygenation of venous blood. On the other hand, when the fish were poisoned with phenolic substances or naphthalene or were dying of dissolved oxygen deficiency, a darkening of the gill color was observed. The field observations on the gill color of dying fish supported the conclusion, based on chemical analyses of the water, that cyanide poisoning was the cause of death. In the laboratory, the bright red color of the gills of salmonid fishes poisoned with cyanide has been observed also by Karsten (1934), who attributed it to the formation of "cyano-hemoglobin", and by other investigators.

SECTION IV

SUBLETHAL TOXICITY OF FREE CYANIDE AND AVOIDANCE REACTIONS OF FISH

EFFECTS ON SWIMMING ABILITY

Neil (1957) found that the swimming ability of brook trout, Salvelinus fontinalis, was impaired very markedly by a month-long exposure to 0.01 mg/l of free cyanide, and even more at concentrations of 0.03 and 0.05 mg/l. The average duration of swimming at a given speed in a rotating, annular chamber (at 9° C) was reduced by about 75, 90, and 98 percent, respectively, at these levels; it was reduced by about 65 and 95 percent after exposure to the highest concentration (0.05 mg/l) for only 21 minutes and 1 day, respectively. Trout exposed to the latter concentration for 35 or 40 days in a continuously renewed medium (KCN solution) showed some improvement of swimming performance at once after return to clean water, but not much additional improvement was apparent after 4 days, and the mean swimming time was about 80 percent of that of controls (which averaged about 25-26 minutes) even when the cyanide-exposed fish had been held in uncontaminated water for 24 (or 20?) days.

Broderius (1970) confirmed these findings in experimenting with young coho salmon, Oncorhynchus kisutch at 15° C. He exposed the fish to the same three cyanide concentrations in continuously renewed NaCN solutions for 2 to 194 hours. He then determined the average duration of their swimming in a tubular chamber against a current of high velocity (which was resisted by controls for about 8.7 minutes, on the average) both in the presence of cyanide and after return of the fish to clean water for varying periods ranging from 6 to 337 hours. After only 2 hours of exposure to cyanide, the impairment of the swimming performance of the fish was nearly or quite maximal, changing little thereafter until the exposure was discontinued. The reductions of swimming time were not quite as great as those reported by Neil for the brook trout but were nevertheless striking, ranging from 56 percent (at 0.01 mg/l CN) to 84 percent (at 0.05 mg/l CN) after only 2 hours of exposure to the cyanide solutions. Recovery of the swimming ability of fish exposed to the cyanide solutions for 193 hours before return to clean water was slow after some initial improvement, which was shown mostly by the fish exposed to the two higher concentrations. Fish that had been exposed to 0.01 mg/l CN showed little improvement after 251 hours. Even after 337 hours some impairment of swimming ability was shown by the fish from all the tested cyanide concentrations, especially the two higher ones. Unfortunately, no tests were performed by either Broderius or Neil at cyanide concentrations below 0.01 mg/l as CN, so that the minimum decidedly effective concentration is unknown.

Leduc (1966) studied the influence of cyanide (NaCN) on the swimming performance of juvenile cichlids, Cichlasoma bimaculatum (L), at 25° C. The fish were exposed to the tested levels of free cyanide for about one month before the swimming performance tests and during these tests. The observed effects of low cyanide concentrations on the swimming ability of the cichlid were not nearly as great as the effects observed in the experiments with salmonid fishes reported above. Even at a free cyanide concentration of 0.04 mg/l as HCN, there was no evident effect or only a moderate effect (a reduction by about 30 percent) on the average duration of swimming against currents of four constant, high velocities, which were resisted by controls for about 2, 4.5, 13, and 26 minutes, on the average. The maximum swimming speed sustained in tests in which the current velocity was raised by degrees at 10-minute intervals apparently was reduced by less than 10 percent, on the average, at this concentration and was not reduced appreciably at a concentration of 0.02 mg/1 as HCN. The effects of these concentrations on the average swimming time at the constant water velocities decreased markedly as the velocities increased, no effect being apparent at the highest velocities tested. At free cyanide concentrations of 0.09 to 0.10 mg/1 as HCN, the effects on swimming ability were more pronounced, but the swimming times at the four constant, high velocities tested were reduced by not much more than 50 percent, on the average (45-67%), and the maximum sustained swimming speeds by only about 25 percent. These concentrations are not far below levels that are lethal for the young cichlids at the experimental temperature of 25° C, the 48-hour median tolerance limit being about 0.14 mg/1 as HCN (Brockway, 1963).

In preliminary experiments with the same fish (juvenile cichlids) exposed to cyanide for 30 days before the swimming performance tests but not during these tests, Brockway (1963) obtained results similar to Leduc's. The duration of swimming against a current of moderate (lowest tested), constant velocity was markedly reduced by previous exposure to cyanide concentrations of 0.056 and 0.10 mg/1 as CN, but not 0.02 mg/1, as compared with that of controls. Only exposure to the highest concentration tested (0.10 mg/1) had an appreciable effect on the duration of swimming at three higher velocities.

The observed effects of sublethal cyanide concentrations on the swimming performance of fish, found in the experiments with salmonids to be surprisingly lasting, may not be disregarded, but the ecological significance of the findings is uncertain. Fish often must swim as fast as they can for a very short time in escaping their enemies or pursuing their prey, but most of them may never need to do so for long periods, even periods as long as a minute. It has been noted that the maximum duration of very rapid swimming of the cichlids was not appreciably affected at moderate cyanide concentrations far below lethal levels. And the large reductions of the maximum duration of swimming at the high and lower velocities of cichlids exposed to relatively high cyanide concentrations were accompanied by only moderate (less pronounced) reductions of the maximum swimming speed sustainable for 10 minutes. Effects of cyanide on the different kinds or indices of swimming ability of salmonid fishes, whose ability to swim at a very high speed and whose maximum sustained swimming speeds after exposure to cyanide were not evaluated, may well be similarly related. It is not obvious or necessarily true that a fish whose ability to swim for a long time at a speed well below its maximal swimming speed, but not for short periods at nearly maximal speeds, has been somewhat impaired is at a serious disadvantage in its natural environment.

EFFECTS ON GROWTH, FOOD CONSUMPTION, AND FOOD UTILIZATION

Leduc (1966) studied the influence of free cyanide (0.008-0.10 mg/l as HCN) also on the growth, food consumption, and food conversion efficiency of the juvenile cichlids, Cichlasoma bimaculatum, which were held in troughs with continuously renewed NaCN solutions at 25° C and with unlimited supplies of live food (tubificid worms). He found that at free cyanide concentrations above 0.06 mg/l and as high as 0.09-0.10 mg/l as HCN, the growth rate was markedly depressed, as compared with that of controls, during the first 12 days of his 36-day experiments. However, the initially pronounced adverse effect on growth was less pronounced during the next 12 days of the experiments, and the growth was faster than that of the controls during the final 12 days. Consequently, there was but little effect on the over-all weight gain in the course of the entire 36-day experimental period, and it appears that, had the experiments been continued for a longer period, no adverse effect at all probably would have been revealed by the total weight gains recorded at the end of that period. At much lower cyanide concentrations, growth tended to be somewhat faster than that of controls at the beginning of the experiments, but slower than that of controls during the final 12 days of the tests. The over-all weight gains of the experimental fish at these concentrations during the entire 36-day tests were not consistently smaller or greater than those of controls, neither retardation nor stimulation of growth having been a predominant effect of the cyanide. The observed change of the response to the high concentrations suggests acclimation to cyanide, but the indicated, quite different responses to very low concentrations are not readily explainable.

At the high cyanide concentrations, the efficiency of food conversion was consistently reduced at all times, whereas the rate of food consumption was usually higher than that of controls held in uncontaminated water, and this difference tended to increase as the experiment progressed. The increase of food consumption effectively compensated for the marked impairment of conversion efficiency, and it must have been the reason for the improvement of growth seen after the fish had been exposed to the cyanide solutions for some time. At low cyanide concentrations, food consumption curiously tended to be reduced slightly during the last 12 days of the experiments, but the amounts of food consumed during the entire experimental periods tended to be slightly greater than those consumed by controls. Each food conversion ratio (weight gain / weight of food consumed) for the entire experimental period was slightly less than that determined for the controls.

Under natural conditions, in the absence of an unlimited supply of food that can be obtained with little or no effort, an increase of food consumption compensating for a reduction of food conversion efficiency is not usually possible. Reduction of activity in the presence of cyanide may even result in a reduction of the amount of food that can be consumed. The need for experiments in which the food supplies are uniformly restricted in evaluating effects of cyanide on growth, and especially for experiments in which feeding is more natural (less effortless) than it is in small aquaria stocked with an abundance of easily found and captured food organisms, thus is clearly indicated.

In a single 24-day experiment with juvenile coho salmon, *Oncorhynchus kisutch*, at 16° C, Leduc (1966) found that salmon exposed in large bottles to free cyanide (NaCN) concentrations of 0.02 to 0.08 mg/l as HCN grew somewhat faster than controls did during the second half of the experiment. Only at the highest one of these concentrations, and not at 0.02 and 0.04 mg/l, was the mean weight gain after 24 days considerably less than that of the controls, because of initial impairment of growth, which was noted also but was less pronounced at the lower concentrations. Adaptation to cyanide is indicated by the improvement of growth after the initial impairment. The food conversion ratio and not the food intake became higher than that of the controls. As in the experiments with cichlids, the test solutions were renewed continuously and the food supply (earthworms) was unlimited. The higher test concentrations of free cyanide (0.04 and 0.08 mg/l as HCN) are not very far below the lethal level (about 0.10 mg/l as HCN) for juvenile coho salmon, and they are about 4 to 8 times as great as a level (0.01 mg/l as CN) that has been shown to have a dramatic effect on the swimming performance of these fish (Broderius, 1970). It appears, therefore, that growth rate is not a very sensitive indicator or measure of cyanide poisoning of juvenile salmonids, as well as of the somewhat more tolerant cichlids tested by Leduc. The swimming performance of the salmonids is clearly a more sensitive indicator or measure, but, as noted already, it is not clear how important relation to the success of the animals in their natural environment are the observed effects of low cyanide concentrations on their swimming ability. Their spontaneous and feeding activities may well be depressed also.

In two of Leduc's experiments with the cichlids, Cichlasoma bimaculatum, in which the growth of the fish was most rapid, body proportions were found to have been altered by prolonged exposure of the animals to the higher cyanide levels tested. An excessive increase of the relative depth and width of the body and caudal peduncle suggested impairment of growth in length. Also, the fins of the fish that had been exposed to the highest tested free cyanide concentrations (0.09-0.10 mg/l as HCN) were found to be abnormally brittle. No pronounced effect of exposure to cyanide on the fat content of the bodies of the fish was apparent.

EFFECTS ON EMBRYONIC DEVELOPMENT, RESPIRATION, AND HEART BEAT

Several physiological studies having to do with effects of sublethal cyanide poisoning on fish embryos, but not directed toward the determination of maximum concentrations of free cyanide that are entirely harmless to the embryos, have been reported a long time ago. The already mentioned resistance of fish embryos to relatively high concentrations of free cyanide may be one reason for the apparent dearth of published information concerning limits of contamination of water with this toxicant compatible with successful embryonic development and hatching. More information on this subject is clearly needed.

Philips (1940) found that the rate of oxygen consumption by embryos of the mummichog, Fundulus heteroclitus, exposed to M/1000 NaCN solutions in sea water (26 mg/l as CN) was maximally reduced within 1 hour. The remaining respiration, which amounted, during the first 6 hours after fertilization of the eggs, to about 32 percent of the average normal respiration, was considered to be a cyanide-stable or cyanide-insensitive portion of the normal respiration. The absolute value of this portion of the normal respiratory rate increased only slightly during the first day after fertilization and remained nearly constant thereafter for 3 additional days of observation. During this time, the total, normal respiration increased greatly as development proceeded, this increase obviously having been due to a progressive increase of the cyanide-sensitive portion. Development of the poisoned embryos did not cease, however, as soon as the respiratory rate was reduced to the minimal level. Embryos whose exposure to the highest tested cyanide concentration (M/1000) began soon after egg fertilization attained late blastula stages or the beginning of gastrulation before the development was completely inhibited, and the rate of their development was not much slower than that of controls. At lower concentrations of cyanide, development of surviving embryos proceeded to later embryonic stages; at M/8,000 and M/16,000 concentrations (3.25 and 1.6 mg/l as CN) it continued after more than 2 days of exposure, but became slower than that of controls. Thus, mummichog embryos proved capable of extensive development, at least before the end of gastrulation, at high concentrations of free cyanide. In M/2000 solutions, embryos whose exposure to the cyanide was postponed continued developing for a shorter time but attained a more advanced developmental stage before development ceased than did those first exposed soon after egg fertilization. Embryos whose development had been arrested were able to continue developing after return to clean sea water. The pH of all the experimental NaCN solutions used by Philips was adjusted to that of normal sea water by the addition of hydrochloric acid (HCl), and experimental temperatures were 20-25° C.

Pelagic eggs (embryos) of marine fishes such as the cunner, Tautogolabrus adspersus, were found by Philips to be more sensitive to free cyanide than were those of the mummichog. At a concentration of M/10,000 (2.6 mg/l as CN), development of cunner embryos proceeded almost not at all beyond the initial, two-cell stage, and even at the concentration of M/40,000 (0.65 mg/l as CN) attainment of early developmental stages was delayed, and the development was arrested in some 4-5 hours, when an early high blastula stage had been reached. Complete inhibition of development at these high concentrations was followed within a few hours by disintegration of the embryos. At the lower concentrations of M/80,000 and M/100,000 (0.325 and 0.26 mg/l as CN), early development was not retarded and later development was not arrested during the 27-hour observation period. However, there was delay of attainment of advanced developmental stages. Apparently, development in the M/80,000 solution had been retarded by about 7 hours by the end of the observation period, when the embryos in that solution extended about half way around the yolk. At the same time, the embryos in the M/100,000 solution and in normal sea water extended about two-thirds and five-sixths of the distance around the yolk, respectively. Even the lowest of the tested concentrations is not, of course, a very low one, as compared with levels lethal to sensitive, fully developed fishes.

Fisher and Ohnell (1940) observed that the frequency of beating of the hearts of mummichog, Fundulus heteroclitus, embryos exposed to M/1000 and more dilute NaCN solutions decreased within about 2 hours to different, constant levels. They considered only the lowest constant frequency attainable by increasing the cyanide concentration as representing a truly cyanide-stable or cyanide-insensitive portion of the over-all, normal frequency. These authors found 72 percent of the normal heart beat frequency of mummichog embryos of unspecified age to be cyanide-sensitive, and 28 percent to be cyanide-stable, being virtually unaffected even by a fourfold increase of cyanide concentration beyond M/1000 (26 mg/l as CN), the minimum level that was said to be necessary to reduce the frequency by 72 percent. The test solutions were prepared by diluting with distilled water a solution of NaCN nearly neutralized with HCl, and were at temperatures of 22 to 24° C.

The highest concentration causing no appreciable reduction of the heart rate was not determined, but could well have been as low as 10^{-5} M (0.26 mg/l as CN).

Armstrong and Fisher (1940) found that 68 percent of the normal heart beat frequency of embryos and young larvae (sac fry) of the Atlantic salmon, Salmo salar, was cyanide-stable. The embryos used in the tests were apparently all approaching the hatching stage, for the authors stated that "if hatching had not yet occurred naturally, the egg membranes were removed to facilitate examination of the heart." The observations were made at a temperature of 6° C and pH 7.1. The highest cyanide concentration having no effect on the cardiac rhythm again was not determined, but clearly was far below 10^{-5} M. The authors stated that "the greatest reversible inhibition of the frequency which can be produced by cyanide does not result in complete stoppage of the heart." This statement is not highly instructive and its meaning is not very clear. It seems to imply that an irreversible reduction of the heart rate by more than 32 percent (the portion reported to have been cyanide-sensitive) and leading to complete stoppage of the heart could be produced by exposure of the test animals to cyanide, but the lowest cyanide concentrations causing the maximum reversible (32%) inhibition and the greater, irreversible inhibition and stoppage of the heart beat were not reported.

Brinley's (1930) data pertaining to mummichog, Fundulus heteroclitus, embryos indicate that at a very high concentration of KCN (1/10 M), stoppage of the heart, which occurred after exposure for about 1 hour, was not preceded by any marked reduction of the rate of heart beat. The rate decreased to decidedly lower levels at somewhat lower concentrations of KCN, at which the heart continued to beat for much longer periods (4-7 hours). The pH of Brinley's test solutions, which were prepared with slightly alkaline sea water (pH about 8.2) was not adjusted, and the most concentrated KCN solutions were said to have been strongly alkaline; their unduly high pH may well have influenced the test results. It is evident, however, that the time to stoppage of the heart can be independent of, or can even vary inversely with, the heart beat frequency at the time immediately preceding the stoppage. The cessation of beating of the heart observed by Brinley at high cyanide concentrations was not irreversible. Within about half an hour to 4 hours after return to clean sea water of exposed embryos whose hearts had ceased beating for as long a period as 60 to 180 minutes, the heart beat was resumed, the time required for this recovery increasing with increase of the cyanide concentration to which the embryos had been previously exposed.

TESTS FOR OTHER NONLETHAL INJURY

In experiments on the influence of free cyanide (NaCN) on the behavior of cichlids, Cichlasoma bimaculatum, in laboratory aquaria, Brockway (1963) observed no effect on the reproductive activity of adults nor on the schooling and fright reactions of juvenile fish at concentrations of 0.02 and 0.10 mg/l as CN. At the higher one of these two test concentrations, which is not far below the 48-hour median tolerance limit for the juveniles, determined to be about 0.135 mg/l as CN at the experimental temperature of 25° C, the young fish were, however, found to feed with less vigor and to consume food slower than did the controls. At the lower concentration tested, this effect was not seen.

Brockway performed some preliminary experiments on effects of chronic cyanide (NaCN) poisoning also on enzyme activities in liver tissues of the cichlids. His results indicated reduction of cytochrome oxidase and succinic dehydrogenase activities by about 10 and 70 percent, respectively, and increase of aldolase activity by some 263 percent in liver tissues of cichlids that had been exposed for 60 days to a concentration of 0.10 mg/l as CN. However, a subsequent, more thorough investigation by Leduc (1966) revealed no simple relations worthy of note between cytochrome oxidase, aldolase, or peroxidase activities in liver tissue homogenates and the cyanide concentrations (nil and 0.02 to 0.1 mg/l as HCN) to which the cichlids had been exposed for periods of 1 to 36 days. Some large variations of the enzyme activities that were observed are not easily interpreted because of lack of consistency. Succinic dehydrogenase activities were not evaluated. Considerable increases of the proteolytic activity of homogenates of intestinal tissues of cichlids exposed to cyanide were observed. The proteolytic activity values tended to increase fairly consistently with increases of both the exposure concentrations (to 0.10 mg/l as HCN) and the duration of exposure (to 20 days). There may well be some connection between these increases of digestive enzyme activity and the relatively high food consumption rates of cyanide-exposed cichlids that have been already reported.

Leduc (1966) also determined changes in body weight and composition of unfed cichlids exposed to cyanide (NaCN) levels of 0.02, 0.04, and 0.09 mg/l as HCN and of unfed controls. Comparison of the changes observed after 6, 12, and 24 days of starvation revealed a marked acceleration of the loss of energy reserves (computed caloric values) in the presence of cyanide. This effect was most pronounced at the highest cyanide concentration tested and after only 6 days of starvation, when the total or cumulative loss (in kilocalories per gram of mean, dry body weight) at the highest concentration was found to have been about four times the control value. After 12 days of starvation at that concentration, however, the cumulative loss was computed to have been less than twice the control value, and the difference in cumulative loss of energy reserves of the experimental and control fish had virtually disappeared after 24 days of starvation. The differences observed early in the experiment strongly indicate loss of metabolic efficiency of the cyanide-poisoned fish, as did also Leduc's data on food conversion already reported. The significance of the subsequent disappearance of the differences is not entirely clear, but it can be attributed to adaptation and probable reduction of activity of the poisoned and starved fish, whose metabolic rates evidently declined markedly during the last 12 days of the experiment.

In tests of short duration (100-155 minutes), Jones (1947) noted pronounced reductions of the rate of oxygen consumption of threespine sticklebacks exposed to NaCN solutions at 17° C and pH 7.0 but not yet overcome by the poison. In 3-4 x 10^{-5} M solutions (i.e., at concentrations of 0.78-1.04 mg/l as CN), average oxygen consumption rates lower than normal rates by 45-68 percent were reported to have been observed after 90-155 minutes of exposure. The 4 x 10^{-5} M concentration, tolerated for 100 minutes before conclusion of the test, was said to be a "critical" one, but even the 3 x 10^{-5} M concentration, tolerated indefinitely by few if any species of fish, actually is far above the minimum lethal level for the threespine stickleback according to the data of Costa (1965). Opercular or breathing rates of Jones' sticklebacks exposed to the NaCN solutions increased at first but later fell to values below the normal values as the oxygen consumption rates continued to decline. Increases of the opercular rate of fish exposed to cyanide solutions have been noted also by Ishida (1947). Remarkably rapid recovery upon return to clean water of fish that had almost ceased breathing in rapidly lethal cyanide solutions was noted by Jones, as it had been also by Karsten (1934) in his experiments with trout.

Carter (1962) reported that, in 300-minute tests, the oxygen consumption of fingerling brown trout that had been confined in sealed bottles until they died was found to have been measurably reduced at cyanide concentrations as low as 0.025 mg/l. Negilski (1973), using a fairly refined technique of oxygen uptake measurement (continuous-flow respirometers), found that the oxygen consumption rate of juvenile chinook salmon, Oncorhynchus tshawytscha, was depressed by about 31 percent, on the average, in the presence of 0.02 mg/l of free cyanide as CN, or about one-fifth of the 96-hour median tolerance limit for these fish. However, the measurements were few, their results highly variable, and the differences between the mean values for the experimental and control fish not highly significant statistically (probability level 0.83). Brockway (1963), using essentially the same method, was unable to demonstrate a definite effect of free cyanide concentrations ranging up to 0.08 mg/l as CN on the also highly variable oxygen consumption rates of his juvenile cichlids, Cichlasoma bimaculatum.

Negilski's (1973) observations on the direct or indirect influence of free cyanide on the food supply and the production of juvenile chinook salmon in artificial streams with model animal and plant communities set up in the laboratory are not very instructive. The desired, constant cyanide concentrations evidently could not be maintained in the circulated water, and the average levels to which the organisms were exposed are not known. Salmon production apparently was increased or reduced somewhat in different tests by the addition of small amounts of cyanide to the water, but, doubtless because of losses of the toxicant, only minute amounts of free cyanide were detected in the stream water by the few chemical analyses that were done. Because the significance of the experimental results is too obscure, they have not been considered in connection with the discussion of the effects of free cyanide on the growth of fish and nothing more will be said about them here.

Chan (1971) found that previous exposure of yearling rainbow trout for 28 days to five free cyanide concentrations ranging from 0.01 to 0.037 mg/l as HCN at 10° C affected their subsequent osmoregulation. There were no noticeable effects on the early phases of adaptation of the fish to a water salinity of 18.9 parts per thousand, but after exposure to the saline medium for 260 hours, the cyanide-poisoned fish were found to have plasma osmotic pressures and chloride concentrations higher than those of controls. When some remaining fish were transferred from the saline medium back to fresh water, again there was no obvious, early effect, but by the end of the test all the cyanide poisoned fish had plasma osmotic pressures and chloride concentrations lower than those of controls. Similar results were obtained when trout adapted for 376 hours to the saline water were transferred to fresh water containing free cyanide at concentrations ranging from 0.01 to 0.037 mg/l as HCN. Histological examination revealed some effect of exposure of the fish for 28 days to 0.037 mg/l of free cyanide as HCN on the epithelial cells of the thyroid gland (5.3 percent reduction of cell height). Exposure of the saline water induced a 60.8 percent increase of the height of epithelial cells of the thyroid follicles in controls, whereas the cyanide-poisoned fish responded to the same treatment by only an 8.6 percent increase of the epithelial cell height.

Hiatt, Naughton, and Matthews (1953b), who observed effects of various chemicals on the behavior of the marine fish Kuhlia sandvicensis during the first 2 minutes of exposure, reported the irritant activity of KCN to have been violent, moderate, and slight at levels of 10, 1.0, and 0.1 mg/l, respectively (4-0.04 mg/l as CN). Elsewhere (Hiatt, Naughton, and Matthews, 1953a), these authors reported only a slight reaction of the same fish to NaCN at a concentration of 1.0 mg/l (0.53 mg/l as CN), but a violent reaction at a concentration of 2.0 mg/l was reported also.

AVOIDANCE REACTIONS

Avoidance reactions of fish to cyanide in a horizontal glass tube, one half of which contained flowing (continuously renewed) tap water and the other half a flowing solution of NaCN (5×10^{-3} N to 1×10^{-6} N) have been studied by Costa (1965). He reported some demonstrable but not sharp avoidance of a concentration as low as 10^{-6} N, or 0.026 mg/l as CN, by young brown trout, which proved most responsive, and also by the minnow Phoxinus phoxinus and the threespine stickleback, but not by young eels (elvers), Anguilla anguilla, nor by young goldfish, Carassius auratus. The sticklebacks showed only a vague and very long delayed reaction to this concentration. Avoidance of 10^{-5} N solutions, or 0.26 mg/l CN, was reported to have been shown by all of the above species, but that shown by the elvers was very slight. Definite avoidance by all the species of 5×10^{-5} N solutions, or 1.3 mg/l CN, was observed. However, the initial avoidance reactions even to much higher concentrations were not immediate, and avoidance of low but still eventually fatal concentrations usually was long delayed. After withdrawing from a dilute cyanide solution, fish often reëntered it and remained in it for some time before withdrawing again.

In Costa's experiments, young individuals generally reacted more slowly than did older and larger fish, but they displayed a greater ability to withdraw from cyanide solutions before being overcome by the poison; only 5×10^{-5} N NaCN solutions were used in the comparative tests with groups of fish of different size. In the presence of a fairly high concentration of sodium thiosulfate (0.025 N), the avoidance by fish of low concentrations of free cyanide was less pronounced or slower than it was in the absence of the antidote. Prolonged prior exposure of fish to thiosulfate also rendered them less responsive to the low cyanide levels. The reactions of threespine sticklebacks to cyanide were more rapid at high temperatures than at low temperatures. Reduction of the pH of a 5×10^{-5} N NaCN solution to pH 5.5 or less by addition of HCl reduced the reaction time and the amount of time spent by the sticklebacks in the solution, as did also reduction of the dissolved oxygen content of the solution. Progressive increase of the pH of the solution to different values up to 10.1 by addition of NaOH progressively delayed and reduced or completely eliminated the avoidance reactions; however, at pH 11.3, the reactions were more rapid and pronounced than they were at pH 10.1 and much like those observed when the pH of the solution was 9.1. Since the pH of the cyanide-free water was not altered in these experiments as was that of the cyanide solution, it was not possible to distinguish between reactions to cyanide and reactions to the pH differences themselves. Avoidance of the solutions of very low and very high pH may well have been mostly avoidance of the extreme pH values or of high carbon dioxide levels and not of cyanide.

Summerfelt and Lewis (1967) performed experiments with a different kind of apparatus, a trough 6.4 m long and 0.6 m wide divided into six compartments of equal length with five transverse gates that could be simultaneously opened or shut (raised or dropped). With the gates shut, five green sunfish, Lepomis cyanellus, averaging 10.8 cm in length were placed in each compartment and the toxicant was introduced into the compartment at one end of the trough. After 20 minutes the gates were opened, and after 5 or 10 (or 15; statements in the text of the paper are contradictory) additional minutes they were again closed, trapping the fish in the compartments in which they were at that time. The final distribution of the fish in the trough, or among the compartments, which was said to have been uniform in control tests without any chemical added to the water in the trough, then was determined and the degree of avoidance of the toxicant by the fish thus was evaluated. The temperature of the water in the trough averaged about 23° C, the pH averaged 7.2, and the dissolved oxygen concentration was rather low, averaging 4.5 mg/l. Summerfelt and Lewis reported that NaCN "was found to be moderately effective as a repellent at 5 mg/l and to produce an avoidance response at 1.0 mg/l." No response was observed to a concentration of 0.5 mg/l or less. Inasmuch as green sunfish have been reported killed in less than 3 hours at a concentration of 1.0 mg/l (as NaCN) and temperatures of about 12 to 27° C, and in 4 to 6 hours at the concentration of 0.5 mg/l and temperatures of 26 to 28° C (Bridges, 1958), it appears that decidedly lethal levels did not all prove even "moderately" repellent. It should be noted, however, that the reported concentrations were the initial concentrations in the compartment at one end of the trough into which the toxicant was introduced. Since there must have been some mixing and dilution of the solution in this compartment with water from the adjacent compartment, producing a concentration gradient in the trough, the exact concentrations to which the fish responded or that they failed to avoid are not known.

Summerfelt and Lewis also briefly reported a pertinent observation made in the field in connection with the sampling of a fish population by the use of cyanide to poison the fish. They stated that a "0.3-acre (0.12-ha) cove of a lake was screened off with a seine and 1.0 mg/l cyanide was applied opposite the screen." They further stated that the fish of all species present in the area "exhibited a strong tendency to congregate along the screen in proximity to the untreated water." It is not entirely clear just what was meant by the first statement quoted. Probably the chemical NaCN was somehow more or less uniformly distriuted throughout the screened-off cove in amount sufficient to produce a concentration of NaCN (not of cyanide as CN) of 1.0 mg/l in all of the water then in that cove. However, the puzzling statement in question can be variously understood, and one cannot say what free cyanide concentrations the responding fish actually had encountered or been exposed to in the treated water.

From the last-mentioned observation and the results of Costa's experiments, it seems reasonable to conclude that fish can sense the presence of harmful concentrations of free cyanide in their medium before they are overcome by the poison, and they probably are able usually to escape from lethal concentrations present only in the immediate vicinity of waste diffusers or other outfalls (i.e., where the poison is diluted to tolerable levels within a very short distance from the points of discharge). However, cyanide concentrations can increase very gradually with decreasing distance from a source of heavy contamination of water with the poison. In view of the slow and uncertain reactions observed by Costa and by Summerfelt and Lewis in the laboratory, one should not conclude that fish encountering harmful cyanide concentrations in such a gradient will usually avoid fatal exposure to these concentrations by turning and swimming away. When confined in a tube such as that used by Costa, with a rather sharp boundary or narrow transition zone in the middle between contaminated and uncontaminated waters, a fish can respond or behave in one of three ways only. It can remain all or most of the time in a solution with a given, uniform concentration of a toxicant, it can remain all or most of the time in uncontaminated water, or it can move back and forth indiscriminately, spending about the same amount of time in each half of the tube. If it is active, it must turn and reverse the direction of swimming frequently, and it cannot choose to continue swimming in one direction and into progressively increasing concentrations of the poison; a flight reaction without frequent turning can lead only to immediate escape from the unfavorable medium. After a number of excursions into, and sojourns in, the contaminated water, or even a single, highly distressing exposure to such water, the fish may learn to avoid that portion of the tube where it has recently suffered distress. Such avoidance of cyanide in the tube, or similar behavior of fish confined in a trough, does not signify that the fish would not continue swimming away from a zone of clean water or in the direction of gradual increase in concentration of the toxicant until it is overcome by the poison, if it had the opportunity to do so. Effectiveness of cyanide as a "directive stimulus", or its ability to repel fish from large areas of natural habitat polluted with eventually lethal or sublethal amounts of the poison, has not yet been convincingly demonstrated. Ishio's (1965) sketchily reported results of experiments with a gradient tank (concentration gradients), indicating only a 50 percent "frequency of avoidance" by fish of a lethal (10^{-5} M) level of HCN, do not in any way contradict this conclusion (see Doudoroff, 1965).

SECTION V

TOXICITY OF COMPLEX CYANIDES

GENERAL, CHEMICAL BACKGROUND

Simple cyanides of some heavy metals, such as mercuric cyanide, $Hg(CN)_2$, as well as those of alkali and alkaline earth metals, are highly soluble in water. Those of most of the heavy metals likely to be important components of cyanide-bearing wastewaters, however, are only very slightly soluble or almost insoluble, cadmium cyanide, $Cd(CN)_2$, being an important exception. These almost insoluble cyanides, such as those of silver or nickel, AgCN or $Ni(CN)_2$, may be formed when soluble salts of the metals, such as silver nitrate, $AgNO_3$, or nickel sulfate, $NiSO_4$, are combined in solutions with simple, alkali metal cyanides, such as KCN or NaCN. However, they are formed and remain as permanent precipitates only when the amounts of the heavy metals exceed the amounts capable of reacting with the alkali metal cyanides to form certain soluble, complex metallocyanides (double salts), such as potassium silver cyanide, $KAg(CN)_2$, or sodium nickelocyanide, $Na_2Ni(CN)_4$. The ionization or dissociation of these highly soluble complex cyanides yields alkali metal cations and complex metallocyanide anions, such as $Ag(CN)_2^-$ or $Ni(CN)_4^{-2}$, which, in turn, dissociate. The dissociation of the complex ions, unlike the first dissociation mentioned, may be very slight or incomplete; it yields metallic cations and free cyanide ions, and the hydrolytic reaction with water of the cyanide ion deriving from the dissociation yields molecular HCN. The following two equilibrium equations pertain to the dissociation of the nickelocyanide or tetracyanonickelate(II) complex:

$$\frac{[Ni^{+2}]\,[CN^-]^4}{[Ni(CN)_4{}^{-2}]} = K_D = \text{about } 5 \times 10^{-31}$$

and $$\frac{[H^+]\,[CN^-]}{[HCN]} = K_a = \text{about } 5 \times 10^{-10},$$

where K_D is the cumulative dissociation or instability constant for the tetracyanonickelate(II) complex ion, $Ni(CN)_4{}^{-2}$, and K_a is again the ionization constant for HCN. The value given above for each of the constants is an approximation, or rounded average, of reported values deemed most reliable and pertaining to temperatures of 20-25° C. The bracketed symbols again represent molar concentrations or, strictly speaking, activities of solution components. It should be understood that the two equilibria considered above "compete" for the cyanide ion, which appears in both equations, and that both equations must be satisfied when the entire system is at equilibrium.

As the nickelocyanide complex ion dissociates, a preponderant portion of the cyanide ion (CN^-) that is liberated is converted into molecular HCN at the pH values of most natural waters. This removal of the CN^- ion from the system permits the dissociation of the complex to proceed much farther than it could otherwise, until no more HCN can be formed. At the ordinary pH levels, the amount of nickel ion (Ni^{+2}) liberated, in gram atomic weights per liter, is almost equal to, or only slightly greater than, one fourth of the amount of HCN formed, in gram molecular weights (moles) per liter, and thus will be nearly constant as long as the HCN concentration remains unchanged. A reduction of the hydrogen ion concentration by one half (i.e., an increase of pH by only slightly more than three tenths of a pH unit) requires a doubling of the CN^- ion concentration if the molecular HCN level is to remain unchanged, according to the second equation given above. Two raised to the fourth power (see the first equation above) is 16. Therefore, if the molecular HCN level is to remain constant in solutions of the nickelocyanide complex in which the total cyanide present is very preponderantly in the form of the complex (i.e., in slightly alkaline and not exceedingly dilute solutions), the concentration of $Ni(CN)_4^{-2}$ ion must increase about 16-fold (not exactly that much) when the pH value is increased by 0.3. For the silver-cyanide complex ion, $Ag(CN)_2^-$, the corresponding increase is about a fourfold increase (x 2^2).

In nickelocyanide solutions, the $Ni(CN)_3^-$ ion species is not formed in appreciable amounts. When the CN/Ni mole ratio is less than 4.0 (e.g., when nickel ion has been added to a solution of $Na_2Ni(CN)_4$ containing no additional cyanide), the very slightly soluble cyanide of nickel, $Ni(CN)_2$, is formed instead. The complex ion species $Ni(CN)_5^{-3}$, $Ag(CN)_3^{-2}$, and $Ag(CN)_4^{-3}$ are known but are unstable in dilute solutions, and they can be formed and can persist indefinitely only in the presence of much free cyanide. On the other hand, the cuprocyanide ions $Cu(CN)_2^-$ and $Cu(CN)_3^{-2}$ can continue to coexist in dilute solutions at equilibrium in considerable amounts and in the presence of relatively very little or much free cyanide, their relative amounts depending on the CN/Cu mole ratio and other factors.

Referring only to the two equations considered above, one can readily calculate the approximate concentration of molecular HCN that is to be expected at equilibrium in a dilute solution of $Na_2Ni(CN)_4$ with any known total cyanide content and pH, and at a temperature of 20-25° C. The total cyanide level in such a solution that is required for producing at equilibrium any desired level of molecular HCN at a given pH can be estimated likewise. The computed values may not prove entirely correct because of some minor complications or sources of error that are not all fully known or understood, such as an influence on the equilibria of the ionic strength of the solutions and some possible formation of metallic ion species (hydrolyzed nickel species) other than the simple, solvated metal cations (aquonickel). "Apparent K_D" values computed by Broderius (1973) from determined molecular HCN levels in various nickelocyanide solutions of different total cyanide content and pH may be more reliable, or more useful when calculations pertaining to similar solutions are made, than other reported K_D values that thermodynamically may be strictly or more nearly correct.

Calculations pertaining to the dissociation of the monovalent dicyano-argenate(I) complex ion, $Ag(CN)_2^-$, in waters of low chloride content are not obviously more complicated than those considered above. However, for reasons not yet determined, there is somewhat more serious disagreement between the "apparent K_D" values for the ion computed by Broderius (1973) and comparable values otherwise derived (and perhaps inaccurate) that had been previously reported in recent chemical literature. Much greater discrepancies between corresponding values for both the ferrocyanide ion, $Fe(CN)_6^{-4}$, and the ferricyanide ion, $Fe(CN)_6^{-3}$, also are still unexplained, but for reasons to be considered later, this is a matter of academic interest only. It should be understood that Broderius' calculations of apparent K_D values are based upon the assumption of the simplest possible systems, like that to which the foregoing equilibrium equations pertaining to the dissociation of the nickelocyanide complex apply. As indicated already, however, the systems in question actually may be much more complicated. For example, protonated species of the cyanide complexes, such as $HFe(CN)_6^{-3}$ or $H_2Fe(CN)_6^{-2}$, may occur in significant amounts. There is not now sufficient reason to conclude, therefore, that the published, thermodynamic constants (K_D values) for the ferrocyanide and ferricyanide complex ions are grossly erroneous; there are reasons to believe otherwise and to seek in the complexity of the systems involved a different explanation for the striking lack of agreement of these values and Broderius' apparent or formal constants. A tendency of silver ions to combine with chloride ions (which thus compete with cyanide ions for the metal cations) in solutions of high chlorinity introduces another interesting complication to be more fully considered later, together with information on the toxicity of the silver-cyanide complex.

Because of the indicated nature of the stepwise dissociation of cuprocyanide complex ions, calculations of equilibrium levels of the various ion species and molecular HCN in cuprocyanide solutions are obviously much more involved and difficult than those pertaining to the nickelocyanide complex. Constants pertaining to the dissociation of the different, coexisting, complex (cuprocyanide) ion species must be introduced into the calculations, which cannot be adequately dealt with here. When the CN/Cu mole ratio is 4.0, for example, use in the calculations of the K_D value for the $Cu(CN)_4^{-3}$ ion and of only two equations corresponding to those given for equilibria in nickelocyanide solutions with the same mole ratio of cyanide to metal leads to patently wrong answers. Some authors apparently have overlooked the error involved in such improper calculations and have reached incorrect conclusions partly for this reason. Calculations pertaining to systems with CN/Cu mole ratios of 3.0 or 2.0 (Broderius, 1973) also are not simple. It appears that the very stable $Cu(CN)_2^-$ ion can be predominant in dilute solutions with CN/Cu mole ratios well in excess of 2.5 when equilibrium has been attained, and this ion can persist at ratios above 3.0. But even a CN/Cu mole ratio of 2.0 does not ensure that virtually no $Cu(CN)_3^{-2}$ ion and no undissolved cuprous cyanide (CuCN) will be present at equilibrium when CuCN is dissolved in a dilute solution of NaCN.

The reader should understand that the same value for the cumulative dissociation or instability constants of different complex ions does not signify equal stability of the complexes if the mole ratios of cyanide to metal are different. Thus, a K_D of 10^{-21}, or even 10^{-19}, for an ion such as the $Ag(CN)_2^-$ ion signifies high stability of the complex, but a K_D value of 10^{-22} for the $Ni(CN)_4^{-2}$ ion (an erroneous value long accepted by chemists), although smaller, would signify a relatively low level of stability. This is true because in the first equilibrium equation given above, pertaining to the nickelocyanide complex, the value of $[CN^-]$ is raised to the fourth power, whereas in the corresponding equation pertaining to the silver-cyanide complex it must be only squared.

The time periods required for attainment of equilibria upon dilution of solutions of the more stable metallocyanide complexes, and also when different metal salts and free cyanide are combined to form the complexes, are extremely variable. Broderius (1973) determined the concentrations of molecular HCN in various buffered experimental solutions of varying pH and total cyanide and heavy metal content and studied the rates of their change attributable to dissociation or formation of metallocyanide complexes. He found that both the dissociation and the formation of the nickelocyanide complex were rather slow in nearly neutral and acid solutions with low or moderate total cyanide concentrations (50 mg/l or less). Dissociation of the complex already formed by combining concentrated solutions of NaCN and $NiSO_4$ was especially slow. When the relatively concentrated, highly alkaline solutions of the complex were greatly diluted with buffered water, with consequent reduction of pH, more time was usually required for attainment of equilibrium than was required when the salts were added separately to the diluent to form the complex (i.e., were combined in dilute solutions). The difference was most pronounced when the pH and the cyanide concentrations were low. At pH 6.5 and total cyanide concentrations of 0.5 and 5.0 mg/l, dissociation equilibria were attained only after about one week to 10 days. The time required for attainment of equilibrium decreased with increase of pH or of the total cyanide concentration, being apparently directly and linearly related to the percentage of the total cyanide that is present as HCN at equilibrium.

After enough nickel to combine with all of the cyanide present, forming the $Ni(CN)_4^{-2}$ complex, had been added (as $NiSO_4$) to a buffered NaCN solution with a cyanide content as high as 250 to 500 mg/l as CN, the concentration of molecular HCN was found to be curiously low initially and to increase with time until equilibrium was attained. In similar but more dilute mixtures of NaCN and $NiSO_4$, the HCN concentrations always were relatively high initially and decreased with time, as they had been expected to decrease because of progressive formation of the $Ni(CN)_4^{-2}$ complex. The anomalous increase of the HCN concentration in the more concentrated solutions of NaCN combined with $NiSO_4$ was tentatively explained by Broderius as having been probably due to gradual release of cyanide from small amounts of complexes such as the pentacyanonickelate(II) complex ion, $Ni(CN)_5^{-3}$. These complexes were supposed to have been rapidly formed initially, together with the much more abundant and stable $Ni(CN)_4^{-2}$ ion, under the conditions of the experiments in question.

The formation and the dissociation of the silver-cyanide complex, $Ag(CN)_2^-$, were found to be quite rapid, the HCN levels determined within a few hundred minutes after preparation of all tested solutions having closely approximated the equilibrium levels. On the other hand, dissociation of the iron-cyanide complexes, $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$, proved exceedingly slow, storage of some solutions of potassium ferro- and ferricyanide for 140 days or more in the dark having been required for attainment of equilibria. The rate of dissociation of the cuprocyanide complex ion $Cu(CN)_2^-$, like that of the nickelocyanide complex, was found to vary greatly with pH and total cyanide concentration. At a total cyanide level of 5 mg/l and pH 6.5, equilibrium was attained only after about 3 weeks, whereas at a total cyanide concentration of 50 mg/l and pH 7.5, equilibrium was attained in less than 1 day.

The appearance of a precipitate (turbidity) upon the addition of a solution of a metal salt to a cyanide solution does not signify that no free cyanide at all remains in the mixture. Some free cyanide deriving from the dissociation of a cyanide complex can coexist with a precipitated, solid metal cyanide. A heavy metal that forms a complex of relatively low stability can be driven out of the complex by a metal capable of forming a more stable cyanide complex. Thus, when nickel ion is added to a solution containing the zinc-cyanide complex, $Zn(CN)_4^{-2}$ (K_D about 10^{-17}), the zinc may be replaced by nickel in the complex, and the consequent liberation of zinc ion may cause precipitation of zinc cyanide.

The iron-cyanide complexes are very stable in the dark, but are subject to rapid photodecomposition with liberation of the cyanide. Reactions of free cyanide with cupric ion are complicated, involving reduction of copper(II) to copper(I) as well as other intricacies. As noted already, competition for metal ions of cyanide ions and other anions that can combine with the metals can influence the dissociation of metal-cyanide complexes, promoting the liberation of cyanide from the complexes. The chemistry of the complex cyanides obviously is not simple, and it is possible to provide here only a very sketchy and not thoroughly instructive introduction to the subject.

TOXICITY OF THE METALLOCYANIDE COMPLEXES IN GENERAL

Early studies of the toxicity to fish of the metal-cyanide complexes have shown clearly that cyanide combined with heavy metals to form at least some of the more stable complexes is nontoxic or much less toxic than free cyanide. Potassium ferrocyanide, $K_4Fe(CN)_6$, was for a long time considered to be a relatively harmless water pollutant (Ellis, 1937). Later, the possibility of production of enough free cyanide to kill fish by photodecomposition of very small amounts (less than 2 mg/l) of the iron-cyanide complexes was recognized (Burdick and Lipschuetz, 1950). Milne (1950) noted that a high concentration of the nickelocyanide complex (more than 100 mg/l as CN) was not evidently toxic to goldfish in a moderately alkaline medium. He then apparently assumed that complexation of cyanide with any heavy metal would result in effective detoxification of the cyanide, rendering it harmless in any receiving water. Evidently he failed to consider adequately, however, the large differences in stability of the different complexes and the important influence of pH on the dissociation of the complexes in dilute solutions.

Stumm, Woker, and Fischer (1954) observed much reduction of the toxicity of cyanide to minnows, Phoxinus laevis, when it was complexed with nickel, copper, or iron, but not when it was combined with zinc or cadmium. They realized that the very pronounced toxicity of the zinc-cyanide and cadmium-cyanide complexes is attributable to their relatively low stability, and that free cyanide or HCN could have been the most important lethal agent in tested solutions of these and other cyanide complexes. An attempt was made, with some apparent success, to relate effective exposure times to computed HCN concentrations in the various solutions of complex cyanides. However, as Doudoroff, Leduc, and Schneider (1966) later noted, the ionization constant for HCN was not correctly introduced into the computations of free HCN levels. This error compensated for the use in the computations of a value for the dissociation constant of the nickelocyanide complex (10^{-22}) that later proved grossly inaccurate, but because of several sources of error, none of the computed HCN levels were correct. Although a general, inverse relation between the toxicity of the metal-cyanide complexes and their stability was, to some extent, revealed, direct dependence of the toxicity of solutions of the complexes on their free cyanide or HCN content was neither demonstrated nor disproved. At least some of the toxicity of solutions of the more stable complexes that were not very rapidly fatal was vaguely attributed to the presence of free heavy metal ions, but no convincing evidence of the suggested, important role of free metallic cations was presented.

Doudoroff (1956), experimenting with fathead minnows, *Pimephales promelas*, also found the toxicity of the zinc-cyanide and cadmium-cyanide complexes to be very great. He concluded that this high toxicity was ascribable mostly to liberation of virtually all of the cyanide (i.e., nearly total dissociation of the complexes) in very dilute solutions. Complexation of cyanide with nickel, copper, and iron greatly reduced its toxicity, but the toxicity of the nickelocyanide complex was found to vary strikingly with the pH of the solutions and to be quite pronounced (not very much less than that of NaCN) at pH levels as low as 6.5-6.6. The detailed observations on the influence of pH on the toxicity of solutions of the nickelocyanide complex were shown generally to support the hypothesis that this toxicity is ascribable to, or is dependent chiefly on the concentration of, molecular HCN. However, some features of the experimental results could not be adequately explained on the basis of the available chemical information and thus fully reconciled with the above-stated hypothesis. Serious inaccuracy of the then generally accepted cumulative dissociation constant for the nickelocyanide complex (10^{-22}), later demonstrated also by chemical studies of other investigators, was the probable reason advanced by Doudoroff for some of the disagreement between his observations and expectations based on theory. A nearly correct estimate of the constant (about 10^{-30}), derived by computation from the toxicity data, then was offered.

Bucksteeg and Thiele (1957) compared the toxicities to fish (species unknown) of cyanide complexes of zinc, cadmium, copper, and nickel with the toxicity of KCN. Concentration limits for harmful action on fish of KCN, $K_2Zn(CN)_4$, $K_2Cd(CN)_4$, $K_3Cu(CN)_4$, and $K_2Ni(CN)_4$ were said to be 0.1, 0.3, 0.75, 1.0, and 30 mg/l as CN, respectively. Considerable reduction of the toxicity of cyanide upon its complexation with all of the four metals is indicated. The publication is of little value, however, because experimental procedures and conditions, such as test solution pH values, are not reported there. The reported concentration limits could not be very meaningful or reliable even if they were correct values for some particular level of pH, inasmuch as the degree of dissociation and toxicity of a metallocyanide complex can vary greatly with the pH of the water. But some of these limits, such as the inordinately high value of 0.75 mg/l as CN for the cadmium-cyanide complex, also are not at all in agreement with other published toxicity data and with pertinent theoretical considerations.

Using the analytical method of Schneider and Freund (1962), Doudoroff, Leduc, and Schneider (1966) were the first investigators to relate the toxicity of solutions of complex cyanides, as well as of NaCN, to analytically determined levels of molecular HCN in these solutions. The tested solutions of NaCN and of a number of different metal-cyanide complexes, of which the nickelocyanide complex was tested most often, varied widely in both total cyanide content and pH. They were prepared with a stream water. The recorded median resistance (immobilization) periods for bluegills at 20° C were plotted in a graph against the determined molecular HCN levels, and also against the concentrations of total cyanide added in preparing the solutions (expressed as HCN). Generally good correlation was found between the resistance time and the molecular HCN concentration, all but one of the plotted points pertaining to this relationship falling close to a straight line which was fitted to these data. The single exception was a point representing the result of a test of a slightly alkaline solution of the silver-cyanide complex. This discrepant result strongly indicated considerable toxicity of the silver-cyanide complex ion itself, the fish having died almost as soon at an HCN concentration that clearly could not alone have been fatal as they died at a much higher HCN level but the same concentration of the complex (10 mg/l as CN) and a lower pH. Pronounced toxicity of the cuprocyanide complex ion $Cu(CN)_2^-$, now believed to be even more toxic than the $Ag(CN)_2^-$ ion (Broderius, 1973), was not revealed. The tested solutions containing copper were prepared by combining NaCN with cupric sulfate, $CuSO_4$, which react to produce cuprocyanide ions. The mole ratio of cyanide to copper (CN/Cu) is believed to have been well above 2.0 (between 2.0 and 2.5), and much free cyanide was present, masking or rendering unimportant any effects of the less toxic $Cu(CN)_2^-$ ion, even though most of the cyanide present must have been in the form of this complex.

No correlation was seen between median resistance time and total cyanide concentration, except that all the points representing results of tests of NaCN solutions and solutions of the zinc-cyanide and cadmium-cyanide complexes were distributed along the above-mentioned straight line. Proximity of these points to the line is not meaningful, inasmuch as the total cyanide concentrations in the particular solutions in question differed only slightly from the concentrations of molecular HCN.

The findings of Doudoroff, Leduc, and Schneider (1966) did not show the toxicity of the metallocyanide complex ions themselves to be always slight, or the contribution of these ions and of the more toxic heavy metal cations deriving from their dissociation to the toxicity of solutions of complex cyanides to be always negligible. Indeed, pronounced toxicity of the silver-cyanide complex ion was revealed. Nevertheless, these experimental results did lend strong support to the supposition that the acute toxicity to fish of very dilute solutions of complexes often to be found in industrial waste waters is usually due predominantly to the presence of free cyanide liberated by dissociation or decomposition of the complexes in the form of accurately measurable, molecular HCN. Therefore, it now seems reasonable to conclude that the toxicity of waters polluted only with alkali metal cyanides or metallocyanides (complex cyanides), and not additionally with metallic or other toxicants in considerable amounts, can usually be estimated without very serious error by measuring and considering only their molecular HCN content. In this connection, it can be noted that silver is not likely to be wasted in large amounts, and that the CN/Cu mole ratios in metal-finishing (plating) wastes containing cuprocyanide complex ions, except effluents effectively treated for cyanide removal, are usually well above 2.0, as they were also in solutions tested by Doudoroff, Leduc, and Schneider.

Views contrary to the conclusion stated above have been expressed, notably in a brief and incomplete review of pertinent literature by Leschber (1969), whose arguments are not, in my opinion, convincing or well founded. This author cited the findings of Bucksteeg and Thiele (1957), which, as noted above, cannot be judged very reliable; he had been especially impressed by the fact that a cuprocyanide, said to have been $K_3Cu(CN)_4$, proved more toxic than $K_2Ni(CN)_4$. He believed the dissociation of the nickelocyanide complex to be much greater than that of the cuprocyanide complex. Although obviously well aware of the work of Doudoroff, Leduc, and Schneider (1966), Leschber evidently overlooked the fact, stressed by the latter authors, but overlooked also by Blaha (1968), that the value of 10^{-22} for the cumulative dissociation constant of the nickelocyanide ion has been repeatedly shown to be greater than the true value by some 8 to 9 orders of magnitude. He probably, also, seriously underestimated and overestimated amounts of free cyanide and cuprous ion, respectively, present in dilute cuprocyanide solutions at equilibrium when the CN/Cu mole ratio is as great as 4.0. Calculations in which the stepwise dissociation of the cuprocyanide ions is correctly dealt with show that in very dilute test solutions of the indicated composition much of the cyanide but not of the copper must be free. It is apparent that the stepwise dissociation of the cuprocyanide complexes, $Cu(CN)_4^{-3}$ and $Cu(CN)_3^{-2}$, has not been properly considered in any calculations of Leschber (1969) and also of Blaha (1968) and Stumm, Woker, and Fischer (1954), only one dissociation constant having been mentioned and considered as pertinent to the equilibrium in each case.

Upon completion of the studies considered above, a number of interesting questions remained to be answered concerning the apparent toxicity of the silver-cyanide complex ion and the influence of chloride ion on the dissociation and toxicity of the complex, the possible toxicity of cuprocyanide complex ions, the prolonged survival of fish in solutions of the iron-cyanide complexes and recently prepared solutions of the nickelocyanide complex with low pH whose computed equilibrium levels of HCN should have proved rapidly fatal, and so forth. After some further investigation of these matters by Doudoroff (unpublished), the task of answering many of the more important remaining questions was undertaken and successfully accomplished by Broderius (1973). The results of his detailed studies of the chemistry and toxicology of metallocyanide complexes are presented, together with various other pertinent data not yet mentioned or adequately discussed, in the following summaries of available information concerning the acute toxicity of various individual metal-cyanide complexes or groups of similar or closely related complexes. Almost no information on the chronic or sublethal toxicity of the cyanide complexes has been found.

ZINC-CYANIDE AND CADMIUM-CYANIDE COMPLEXES

Doudoroff (1956) and Broderius (1973) have noted that the dissociation constants of the order of 10^{-17} or 10^{-19} attributed to the zinc-cyanide and cadmium-cyanide complex ions, $Zn(CN)_4^{-2}$ and $Cd(CN)_4^{-2}$, do not signify slight dissociation of the complexes in dilute solutions even when the pH is high. On the contrary, computations have shown that, in very dilute but acutely toxic solutions of these complexes, with total cyanide concentrations less than 0.3 mg/l as CN, the dissociation should be almost total at any level of pH commonly encountered in natural waters. Thus, it seems reasonable to conclude that waste cyanide complexed with zinc or cadmium in concentrated, alkaline solutions (wastewaters) should be about as dangerous as free cyanide to fish in receiving waters, if not more dangerous.

Doudoroff (1956) found dilute solutions of the two complexes to be apparently somewhat more toxic to fathead minnows than comparable NaCN solutions with the same total cyanide content. The 96-hour median tolerance limits of NaCN and of solutions of the zinc-cyanide and cadmium-cyanide complexes prepared by combining NaCN with $ZnSO_4$ or $CdSO_4$ and having cyanide/metal mole ratios slightly less than 4.0 were found to be equivalent to about 0.23, 0.18, and 0.17 mg/l of cyanide as CN, respectively. The concentrations of the metals, especially of zinc, in the solutions of the complexes that proved lethal to about 50 percent of the test animals were far below levels tolerated by most of the fish for 96 hours in the absence of cyanide. These results indicate cooperative joint action or synergism (in a broad sense, as explained farther) of free cyanide and zinc or cadmium ion; however, they cannot be regarded as a conclusive demonstration of such interaction, only one toxicity bioassay of NaCN and of each of the two complex cyanide solutions having been performed.

Chen and Selleck (1969) observed a similar interaction of zinc and cyanide in experiments with guppies. They concluded that the "additive nature of the zinc and cyanide toxicities is indicated clearly, i.e., the permissible zinc concentration increases with decreasing cyanide concentration and vice versa." Unfortunately, they also stated that an "antagonistic effect was noted between the zinc and cyanide ions because a mixture of the two ions always has less toxicity than that obtained by the simple addition of the toxicities of the two components determined separately under otherwise identical conditions." This statement reflects not only a lack of recognition of the greater importance as a toxicant of molecular HCN than of cyanide ion, but also, more importantly, an all too common misconception leading to misuse of the term "antagonism", which denotes active opposition, resistance, or counteraction.

The error involved in the system of terminology that equates or confuses strictly additive joint toxicity of two or more combined toxicants (which is merely one kind or level of cooperative action) with a lack of any interaction of the toxicants has been emphatically pointed out by Sprague (1970) and by Warren (1971). As Sprague has explained, this system, adopted by several authors in the past, "fails to cover certain categories of effect adequately. In particular, it ignores the case where each of two toxicants acts as if the other were not present. Such a case ends up by default in the category 'antagonism'. Similarly, two toxicants which have a combined effect somewhat greater than either one separately, but less-than-additive, would be relegated to 'antagonism'. Obviously, the term antagonism, which signifies counteracting or opposing effects, cannot include the case where two toxicants do not affect each other's action nor the case where they work together somewhat."

Warren (1971), who has discussed the terminological problems in more detail, has pointed out also that the term antagonism should be reserved for physiological phenomena and not applied to chemical and physical reactions that occur in the external medium of an organism. The reaction of highly toxic heavy metal cations with free cyanide to form relatively harmless complex ions or precipitates of insoluble metal cyanides in the medium of aquatic organisms is not true antagonism. The effective toxicants having been eliminated from the medium through chemical reaction, they cannot be correctly said to counteract each other or to have opposing effects on the organisms.

Both Sprague (1970) and Warren (1971) prefer to avoid the use of the term synergism because of its ambiguousness. In a broad sense and, in my view, the most correct sense, synergism denotes truly joint or cooperative action at any level, including additive and infra-additive (less-than-additive) interaction, with the exception only of "response addition" (Anderson, 1974) already explained. The term has also been often used, however, in a narrower sense, as a synonym of potentiation, supplemental synergism, or supra-additive (more-than-additive) joint action. It has thus indeed lost much of its usefulness. But the term antagonism also has become ambiguous because of its frequent misuse in referring to infra-additive joint action that involves no counteraction. In my opinion, both terms are still useful when employed correctly and defined clearly, and so they are used in this review.

Even infra-additive synergism of cyanide and zinc and of cyanide and cadmium has not always been observed. Doudoroff, Leduc, and Schneider (1966) found the median survival times of bluegills in solutions of cyanide (0.40-0.64 mg/l) combined with zinc or cadmium (one solution with each metal) to be slightly greater than those predictable on the basis of determined molecular HCN levels, the known toxicity of HCN to the fish, and the assumption that the metals would not contribute to the toxicity of the mixtures. The differences between the latter median survival times and those experimentally determined, which were not long (256 and 134 minutes), were too small to be interpreted as indicating true antagonism of the combined toxicants. They may well have been due simply to unavoidable experimental error, but they do not suggest any synergism or cooperative action of the toxicants. The data of Stumm, Woker, and Fischer (1954) also indicate some reduction of the toxicity of cyanide in the presence of cadmium or zinc, but the concentrations of the two complexes tested by them (10-100 mg/l as CN) were much too high and too rapidly fatal for these results to be very meaningful or instructive.

Bucksteeg and Thiele (1957) reported minimum concentrations harmful to fish of KCN, $K_2Zn(CN)_4$, and $K_2Cd(CN)_4$ to be 0.1, 0.3, and 0.75 mg/l as CN, respectively. These values indicate very pronounced reduction of the toxicity of cyanide in the presence of the two heavy metals in question, especially of cadmium. One can have no confidence in their validity, however, in the absence of sufficient information about their experimental derivation and in view of all the contradictory experimental evidence and theoretical considerations presented above. Doudoroff (1956), whose experiments included some toxicity tests of purified $K_2Zn(CN)_4$, and others have shown that complexation of free cyanide with zinc and cadmium certainly does not render it so much less dangerous to fish in very dilute solutions.

Cairns and Scheier (1968) reported some reduction of the toxicity of cyanide to bluegills when KCN was combined with zinc chloride, $ZnCl_2$, in dilute test solutions. The 96-hour median tolerance limits reported were 0.18 mg/l as CN for KCN alone and 0.26 mg/l as CN for the mixture. The two salts were combined in such proportions that, when the concentration of cyanide (total) was 0.26 mg/l as CN, that of zinc was 3.9 mg/l, a level only slightly below the 96-hour median tolerance limit of the metal ion, determined by testing solutions of $ZnCl_2$ alone and reported to have been 4.2 mg/l. The mole ratio of zinc to cyanide obviously was far in excess of 0.25, the ratio for the $Zn(CN)_4^{-2}$ complex. The authors suggested that the apparent reduction of the toxicity of cyanide in the presence of the added zinc was due probably to complexation. However, there is no evidence that they undertook any calculations to determine how much of the cyanide remained in solution, or that none was present as a zinc cyanide precipitate (i.e., that the solubility product constant for $Zn(CN)_2$ was not exceeded), and then to evaluate the extent of complexation of cyanide and zinc that could have occurred in their test solutions. I have not attempted such calculations, which would have been quite complicated and would have required knowledge of the pH of the test solutions (which was not reported) or the use of assumed values, but I am almost certain that no material complexation of zinc and cyanide was possible at any possible pH of the solutions. The reported toxicity test result may have been due simply to experimental error.

It can be concluded that there is more impressive evidence of synergism of cyanide and zinc or cadmium in very dilute but still acutely toxic solutions of the complexes in question than of their true antagonism, but no interaction of great importance has been demonstrated. Complexation of cyanide with the two metals certainly can be disregarded in establishing maximum permissible concentrations of these toxicants when they are present together in receiving waters in which fish are to be protected. It clearly cannot be important in any solution with a level of free cyanide that can be regarded as safe for fish life, and the contrary finding of Bucksteeg and Thiele (1957) must be discounted.

It can be noted, additionally, that Doudoroff (1956) showed that the introduction into a fairly concentrated, clear solution of the zinc-cyanide complex of enough $NiSO_4$ to produce a small amount of precipitate (by replacement of some of the zinc with nickel in the complex) did not materially reduce the great toxicity of the solution to fathead minnows. This result was to be expected, since most of the cyanide in the solution remained in the form of zinc-cyanide complex after the addition of nickel, and the dissociation of this complex upon dilution of such a solution could not be affected by the presence of a small amount of nickel.

NICKEL-CYANIDE COMPLEX

Milne (1950) found that goldfish were not noticeably affected after exposure for 24 hours to a solution in which NaCN and $NiSO_4$ were combined to form the nickelocyanide complex and whose pH was 8.1. The measured total cyanide content of this test solution was 104 mg/l and the nickel content 77 mg/l, a very excessive amount if both reported values are correct, for they indicate a CN/Ni mole ratio of 3.05 and not near 4.0. The pertinent findings of Stumm, Woker, and Fischer (1954) and of Bucksteeg and Thiele (1961), already discussed, also indicate low or only moderate toxicity to fish of the nickelocyanide complex but are not highly instructive.

Doudoroff (1956) tested for toxicity to fathead minnows at 20° C some nickelocyanide solutions prepared by slowly adding a fairly concentrated solution of $NiSO_4$ in distilled water to a like, agitated solution of NaCN until a small amount of $Ni(CN)_2$ precipitate appeared. The CN/Ni mole ratio was either 3.95 or 3.81 (in one early experiment only). The solutions prepared in the above manner, which were quite alkaline in reaction, were diluted as necessary for the toxicity tests with a synthetic, soft water (total alkalinity 17.5 mg/l as $CaCO_3$; total hardness 25 mg/l as $CaCO_3$). When the dilutions were neither renewed periodically nor artificially aerated, but remained adequately oxygenated through absorption of oxygen at the surface exposed to the atmosphere (dissolved oxygen usually 5 to 6 mg/l after the first 48 hours), the nickelocyanide complex eventually proved highly toxic to the fish. Some of the fish (20-40%) died within 168 hours at a total cyanide concentration of 0.5 mg/l as CN, and the minimum concentrations necessary to kill half of the fish in 96 and 168 hours were estimated to be 0.95 and 0.65 mg/l as CN, respectively. However, at no tested concentration below 600 mg/l as CN were all of the fish, or more than 80 percent, killed within 24 hours. At concentrations of 8 to 67 mg/l as CN, all of the fish died in less than 48 hours, but at the concentration of 200 mg/l as CN, all lived for 24 hours and 80 to 90 percent survived for 48 hours. The result of a single test of a purified potassium nickelocyanide, $K_2Ni(CN)_4$, at the concentration of 2.0 mg/l as CN agreed very well with those of comparable tests of the mixture of NaCN and $NiSO_4$ solutions diluted so that the total cyanide level was the same.

It was soon determined that the toxicity of all the initially more or less alkaline, standing test solutions increased markedly with time after introduction of the test animals. This increase of toxicity was shown to be due mostly to the decrease of the pH of the solutions caused by the production of carbon dioxide, CO_2, by the respiring fish. Periodic renewal of test solutions (i.e., their replacement with fresh solutions to which the fish were transferred), or mere addition to them from time to time of small amounts of sodium hydroxide, NaOH, so as to return the pH approximately to its initial value, greatly prolonged the survival of the fish, or made possible their indefinite survival, at a concentration of the complex that had proved fatal within two days without such renewal or treatment of the solutions. An increase of the pH of an aged test solution by only about half a pH unit upon addition of NaOH was found to be sufficient to bring about prompt recovery of fish that had already been visibly affected (i.e., in distress) in the same medium before the treatment. Dilute solutions proved more rapidly fatal than much more concentrated ones evidently because critical pH values were attained sooner in the former solutions than in the more concentrated and initially more alkaline solutions as the pH of all was reduced by the respiration of the fish.

In a subsequent experiment, Doudoroff used five different waters of varying total alkalinity (synthetic waters resembling natural waters with total alkalinity values of 5 to 192 mg/l as $CaCO_3$) to dilute a concentrated stock solution in which 5,150 mg/l of cyanide as CN had been combined with 2,940 mg/l of nickel (CN/Ni mole ratio 3.95). The pH values and dissolved oxygen concentrations of the dilution waters were reduced to nearly constant levels by holding test animals in jars (test vessels) containing these waters for 2 days before any toxicant was added. The pH was promptly readjusted to these levels, as necessary, by addition of CO_2 after the stock nickelocyanide solution had been added to these waters to make up test solutions of the various desired concentrations. Thus, toxicity tests were performed and the 24-, 48-, and 96-hour median tolerance limits (TL_{50}'s) were determined at each of five different, nearly constant levels of pH ranging from about 6.5 to 8.0 (mean values) and at nearly uniform oxygen concentrations. The 24-hour TL_{50} values so determined, when expressed as initial total cyanide (CN) concentrations, ranged from 1.35 mg/l (at pH 6.5) to 1,300 mg/l (at pH 8.0); the 48-hour TL_{50} values ranged from 0.75 mg/l (at pH 6.5-6.6) to 1,200 mg/l (at pH 8.0); and the corresponding 96-hour values ranged from 0.42 to 730 mg/l. Each curve relating the logarithms of the TL_{50} values for a given exposure period to the mean pH values was sigmoid in shape. The largest increases of log TL_{50} with a given increase of pH occurred in the pH range of 7.4 or 7.5 to 7.8. With increase of pH from 7.5 to 7.8, the TL_{50} values increased about 10-fold to 13-fold. The magnitude of these increases agrees fairly well with that of the (roughly) 16-fold increase in concentration of the nickelocyanide complex in slightly alkaline solutions that is required, according to theory as explained earlier in this review, for maintaining a constant concentration of molecular HCN when the pH is increased by three tenths of a pH unit.

In view of the approximate agreement just mentioned of the maximum slopes of theoretical and experimentally derived curves, Doudoroff concluded that the acute toxicity of dilute and not very alkaline solutions of sodium nickelocyanide must be determined chiefly by their molecular HCN content. The striking influence of pH on the toxicity could not be explained otherwise. However, in the year 1956, it was not possible to reconcile the available toxicity data and the proposed explanation thereof with the then generally accepted value for the cumulative dissociation or instability constant of the $Ni(CN)_4^{-2}$ complex ion, namely, 10^{-22}. Having made the necessary calculations, and assuming that the toxicity of molecular HCN is not materially reduced in the presence of ionic nickel, Doudoroff concluded that this value for the constant, K_D, probably is grossly erroneous and that a value of the order of 10^{-30} would better accord with the toxicity test results. He noted that the tested solutions should have had a stronger odor of HCN than they did, as well as much greater toxicity to fish, if the correct value for the constant were nearly as great as 10^{-22}. Freund and Schneider (1959) thereupon redetermined the constant at 25° C, finding it to be about 1×10^{-31}, but Schneider and Freund (1962) later recomputed it, using a new value for the ionization constant of HCN that was deemed most reliable, and reported a corrected value for K_D of 5.4×10^{-31}. Results in fairly close agreement with these findings and with Doudoroff's (1956) estimate were subsequently reported by Christensen et al. (1963) (indicated K_D 7.9×10^{-31} at 25° C) and by Broderius (1973), whose somewhat variable "apparent K_D" values based on determined HCN levels in various nickelocyanide solutions prepared by him averaged 1.0×10^{-31}.

Complete agreement of Doudoroff's (1956) toxicity data with calculated molecular HCN concentrations in the test solutions at equilibrium evidently could not be achieved by using the correct dissociation constant for the $Ni(CN)_4^{-2}$ ion in the calculations. Outside the pH range of about 7.4 to 7.8, serious discrepancies remained that needed to be explained. The toxicity of relatively concentrated solutions with pH near 8.0 was apparently greater than that which could have been due entirely to their free cyanide or molecular HCN content. On the other hand, the observed toxicity of very dilute solutions that were slightly acid or nearly neutral was much less than that which could have been expected on the basis of the correctly computed equilibrium concentrations of molecular HCN and the assumption that the low-level nickel ion present exerted no material, truly antagonistic effect counteracting the toxic action of HCN.

Doudoroff (1956) observed that some of the ions present in relative abundance in his most concentrated and alkaline solutions could well have contributed materially to their toxicity. The $Ni(CN)_4^{-2}$ ion certainly could not be supposed to be entirely harmless at very high concentrations. As for the fact that the dilute solutions that were neutral or slightly acid proved less toxic than they apparently should have been, Doudoroff was unable in the year 1956 to propose an adequate explanation of it; his explanation was only a partial one. He pointed out that when the fraction of the total cyanide that is free cyanide becomes large, the difference between total cyanide and cyanide bound in complex ions becomes important, i.e., it can no longer be neglected in considering or predicting effects of further reductions of pH on the toxicity of the complex. He further noted that, in the absence of a large reservoir of potential toxicant in the form of complex ions, gradual loss of cyanide from very dilute, acid and neutral test solutions during extended bioassays could have been considerable, augmenting the initial concentrations necessary to render the solutions lethal to fish. But the possibility of important effects of other, unidentified factors also was recognized by him. Only much later did he realize, as reported by Broderius (1973), that equilibria in his more dilute test solutions with relatively low pH may not have been attained for a long time after the tests of the fresh media were begun. He then found that when such solutions were aged by storing them in sealed vessels (without fish), their toxicity increased markedly, approaching the levels expected on the basis of theoretical considerations upon attainment of equilibria. Thereafter, Broderius (1973) undertook many additional experiments designed further to elucidate the significance of the observations reported above.

Broderius' (1973) findings concerning the rates of dissociation and formation of the $Ni(CN)_4^{-2}$ complex ion in solutions of varying pH and total cyanide content already have been summarized briefly. Having determined how much time was required for attainment of equilibria in dilutions of a concentrated nickelocyanide solution, he was able to evaluate both the molecular HCN content and the toxicity to bluegills of dilutions in which equilibria had been nearly or fully attained. When the total cyanide concentrations in the test solutions, with pH 7.1 or 6.5, did not exceed 10 mg/l as CN and the average HCN concentration was 0.20 mg/l, the median survival times of the bluegills (240-257 minutes) agreed well with that of fish exposed to an NaCN solution (pH 7.1) with the same determined HCN content (260 minutes). The solution with pH 6.5 contained initially 0.23 mg/l of cyanide combined with nickel and had been aged for 20 days before it was tested, when the dissociation of the complex ions was believed to have been almost total; a small amount of cyanide initially present in this solution and about the same amount initially present in the NaCN solution apparently were lost during the tests. Nickelocyanide solutions exactly like the last mentioned one (pH 6.5) but aged for only 5 and 8 days had determined HCN levels of only 0.08 and 0.11 mg/l, respectively, and killed no bluegills within a period of 480 minutes, after which the tests were discontinued. The median survival times of bluegills in similar, sufficiently aged (for 1 day) nickelocyanide solutions with determined molecular HCN content 0.20 mg/l but with total cyanide concentrations of 248 and 500 mg/l as CN and with pH values of 7.45 and 7.60 were only 196 and 140 minutes, respectively. A linear relation of median survival time to total cyanide concentration in nickelocyanide solutions with varying pH but a constant molecular HCN concentration is indicated. The indicated, moderate increase of toxicity associated with great increase of total cyanide in solutions of constant HCN content (but different pH) is of little practical importance but is nevertheless noteworthy. It clearly was too pronounced to be ascribed to the increase of the always very low concentration of cyanide ion accompanying the increase of pH to levels not exceeding 7.6, and it could most reasonably be attributed to toxicity and synergistic action of the nickelocyanide ion itself.

Evidence of the ability of the nickelocyanide complex ion to penetrate the tissues of fish exposed to toxic nickelocyanide solutions has been presented by Broderius. He used ^{14}C-labeled cyanide combined with nickel, and he compared the rates of accumulation of the radioactive carbon in tissues of bluegills exposed to solutions containing the labeled complex and to solutions of NaCN alone, also containing ^{14}C-labeled cyanide. The concentrations of molecular HCN in the nickelocyanide solutions were approximately the same as those in the NaCN solutions. Fairly reliable estimates thus could be made of the rates of accumulation of ^{14}C in the tissues of fish exposed to the former solutions that was not a consequence of, or referable to, the intake of HCN. Broderius concluded that the $Ni(CN)_4^{-2}$ complex ion does not enter the fish body readily, but its limited penetration of the tissues of fish exposed to relatively concentrated and alkaline nickelocyanide solutions may well be the reason why the toxicity of these solutions did not appear to be attributable entirely to their molecular HCN content. The ^{14}C accumulated in the gill tissues much more markedly than it did in the blood and in tissues of internal organs sampled.

The experiments of Doudoroff, Leduc, and Schneider (1966), whose methods and findings already have been described, did not reveal any considerable influence of the $Ni(CN)_4^{-2}$ ion at concentrations up to 500 mg/l as CN on the acute toxicity of nickelocyanide solutions. These experiments were quite similar to those of Broderius that led him to the conclusion that the complex ion itself may have considerable toxicity, effective even in tests of short duration, at concentrations of 500 mg/l as CN and less. No explanation can be offered for the disagreement of the results in question, and I cannot say whose evidence is more reliable. Broderius' methods were refined but his pertinent tests fewer than those of Doudoroff, Leduc, and Schneider. Even if Broderius' finding is correct, it certainly does not invalidate the conclusion of Doudoroff, Leduc, and Schneider that reduction of the pH of nickelocyanide solutions (test media) at gill surfaces by carbon dioxide released there did not cause the HCN concentrations there to be materially greater than those in the inspired, ambient media. This conclusion was based mostly on a comparison of median survival times of bluegills exposed to poorly and well buffered, neutral or slightly alkaline nickelocyanide solutions (100 mg/l as CN) of widely varying free CO_2 content, whose variations were not found to have any appreciable effect on the toxicity of solutions of uniform pH. The reasoning and computations that led to this conclusion, which is not deemed of major importance, are too involved to be fully or adequately reported here with the supporting data. The conclusion, which implies that the dissociation of $Ni(CN)_4^{-2}$ ions upon reduction of the pH of a solution cannot be very rapid, is now further supported by Broderius' data on the rates of dissociation of the complex ions. It is apparent that liberation of ^{14}C-labeled cyanide at the gill surfaces was not the reason why some of the labeled cyanide present in test solutions in the form of nickelocyanide ions, and not as molecular HCN, was evidently able to penetrate the tissues of bluegills in the experiments reported by Broderius. Penetration of the gills by the complex ions themselves apparently did occur.

Doudoroff (1956) observed that the bodies of fathead minnows exposed to fairly concentrated nickelocyanide solutions that were alkaline enough not to be rapidly lethal to the fish became often, but not invariably or with any apparent regularity, exceedingly swollen some time before the fish died, perhaps of osmoregulatory failure. Red blotches, apparently due to internal hemorrhages, commonly appeared on the abdomens of the fish so affected. No such signs of intoxication were observed in any fish dying, apparently of HCN poisoning mainly, in test solutions that were more dilute than the above-mentioned ones but were about as toxic because of their lower pH. A distinctive kind of subacute poisoning with penetrating nickelocyanide ion of the fish exposed to high concentrations of the ion thus is suggested by the reported, curious observations.

The practical significance of the reviewed information on the toxicity of the nickelocyanide complex, whose acute toxicity to fish has been studied more intensively than that of any other metal-cyanide complex, will now be considered. Complexation of free cyanide with nickel obviously can be very effective in protecting fish from lethal effects of the cyanide in alkaline waters (e.g., at pH near 7.5), but not at all effective in decidedly acid waters (at pH 6.5 or less) in which HCN levels near equilibrium levels can be attained. In very dilute solutions with pH near 6.5 or less, the dissociation of the complex that tends to render the solutions acutely toxic is very slow, however; less than one third of it may take place during the first day. If the cyanide gradually liberated at the low pH in the form of volatile HCN is rapidly lost to the atmosphere or otherwise, as it may very well be lost from even moderately turbulent waters of receiving streams, even a fairly high initial concentration of the complex may never become fatal to fish. But let us assume that virtually no liberated cyanide will be lost to the atmosphere or otherwise eliminated during the time period required for attainment of equilibrium. In that case, any dilution of the complex with so low a pH (6.5 or less) and with a cyanide content great enough to kill sensitive fishes when the cyanide is all in the free state will eventually become lethal to the fish. The dissociation of the complex ion will be virtually total, or, if the initial concentration of the complex and the pH are not low enough for such complete dissociation to take place, the dissociation will be more than sufficient to render the water acutely toxic to fish. In alkaline waters, on the other hand, total cyanide levels many times the minimum lethal concentration of free cyanide may be necessary to render the waters acutely toxic, even when no cyanide is lost.

We must also consider, however, just how well complexation of free cyanide with nickel can protect sensitive fishes in alkaline waters against the harmful effects of extremely low, sublethal concentrations of free cyanide. My calculations show that it can have very little effect or be wholly ineffective even when the pH of the water receiving the complex remains at a level as high as 8.0, if there is no rapid loss of cyanide. For example, let us assume that the highest concentration of molecular HCN that would have no serious adverse effect on a sensitive species of fish inhabiting a given water with the pH of 8.0 is only 2×10^{-7} moles per liter, or about 0.005 mg/l. We have seen that as little as 0.01 mg/l of free cyanide (mostly HCN) has a speedy, very pronounced, and lasting effect on the swimming ability of salmonid fishes; thus, concentrations even well below 0.005 mg/l may be shown by further research to be decidedly harmful to these fish. The latter concentration has been considered or proposed by some authorities as a reasonable upper limit for permissible free cyanide concentrations in waters in which valuable fishes are to be well protected. Substituting the appropriate values (rounded approximations) in the pertinent ionic equilibrium equations already presented and explained, after computing (solving for) the values of $[CN^-]$ and $[Ni(CN)_4^{-2}]$, we get:

$$\frac{[H^+]\,[CN^-]}{[HCN]} = \frac{[10^{-8}]\,[10^{-8}]}{[2 \times 10^{-7}]} = 5 \times 10^{-10} = K_a, \quad \text{and}$$

$$\frac{[Ni^{+2}]\,[CN^-]^4}{[Ni(CN)_4{}^{-2}]} = \frac{[5 \times 10^{-8}]\,[10^{-8}]^4}{[10^{-9}]} = 5 \times 10^{-31} = K_D,$$

since the ionization of HCN at pH 8.0 is slight, and so $[Ni^{+2}]$, which should be equal to $([HCN] + [CN^-])/4$, can be taken to be simply equal to $[HCN]/4$. It can be seen from the first one of these equations that when the concentration of molecular HCN is 2×10^{-7} moles per liter (0.0052 mg/l as CN) and the pH is 8.0, or $[H^+]$ is 10^{-8}, the concentration of the CN^- ion must be about 10^{-8} also (0.00026 mg/l). From the second equation, it can be seen that, if all of the free cyanide (HCN and CN^-) derives from dissociation of the $Ni(CN)_4{}^{-2}$ complex ion, and the value for the dissociation constant (K_D) of this ion that has been used above (5×10^{-31}) is very nearly correct and applicable, then the concentration of the undissociated $Ni(CN)_4{}^{-2}$ ion must be about 1×10^{-9} moles per liter, or equivalent to about 4×10^{-9} moles of cyanide (CN) per liter (0.0001 mg/l). Thus, of the total cyanide (about 0.0056 mg/l as CN), less than 2 percent must be present in the form of the complex ion at equilibrium under the specified conditions, the remainder being present as free cyanide, and most of this (nearly 95%) as molecular HCN. In other words, the dissociation of the complex must be almost total.

If the cumulative dissociation constant of the tetracyanonickelate(II) ion is now taken to be as low as 1×10^{-31}, the average of "apparent K_D" values computed by Broderius (1973) from determined HCN concentrations in his various nickelocyanide solutions at 20° C, the result of the calculation in question is not seriously altered. Computations then show that about 9 percent of the total cyanide should be bound in complex ions at pH 8.0, but only about 1 percent at pH 7.7, when the HCN concentration is 2 x 10^{-7} moles per liter. The pH values of most fresh, surface waters receiving waste cyanides are not much above 8.0. Complexation of free cyanide with nickel before discharge evidently cannot be expected to prevent the occurrence in these waters of free cyanide levels above 0.005 mg/l when the levels of total cyanide introduced in the form of the complex considerably exceed 0.005 mg/l and persist until equilibria are fully or nearly attained. The addition of nickel ion to moderately alkaline waters seriously polluted with free cyanide obviously also is not likely to render these waters entirely suitable for sensitive fishes, though it often could reduce the free cyanide concentrations to levels that these fishes can tolerate. It is appropriate to remark here that, when the possible effectiveness of complexation of cyanide with nickel as a means of preventing the occurrence of lethal levels of free cyanide in nearly neutral waters is evaluated, the choice of values to be used in calculations for the dissociation constant of the complex and the HCN tolerance limit of fish to be protected becomes critically important. Use of different values whose differences may seem to be minor and probably unimportant can lead to very different conclusions.

SILVER-CYANIDE COMPLEX

Doudoroff, Leduc, and Schneider (1966) observed that the toxicity to bluegills of a somewhat alkaline solution (pH 7.5) containing 10 mg/l of cyanide (CN) complexed with silver was not much greater than that of a similar but slightly acid solution (pH 6.5) with the same total cyanide content. The determined molecular HCN concentration in the former solution was only 0.02 mg/l, whereas the concentration in the slightly acid solution was 0.12 mg/l. The median resistance times of the bluegills exposed to the two solutions were nearly the same (833 and 789 minutes), and so were evidently almost independent of the HCN concentrations. A similar solution with the same total cyanide content (10 mg/l as CN) but a pH of 6.0 and a determined molecular HCN content of 0.19 mg/l was much more rapidly fatal, and its greater toxicity was evidently attributable entirely or almost entirely to the high HCN concentration. In the solutions with higher pH, the dying fish often showed no signs of cyanide poisoning. Pronounced, superficial coagulation of mucus suggestive of heavy-metal poisoning was usually observed in fish that had been exposed for some time before death to the solutions containing the silver-cyanide complex. These observations indicated that the $Ag(CN)_2^-$ ion itself, unlike the $Ni(CN)_4^{-2}$ ion, has fairly high toxicity to fish. The possibility that the extremely toxic silver ion present in small amounts because of dissociation of the complex caused or contributed to the toxicity of some of the tested solutions (at pH 6.5-7.5) was considered, but an important role of this toxicant was not demonstrated. Also considered was the probability of increased dissociation of the complex and production of HCN because of combination of the silver ion with chloride ion, which was fairly abundant in the acid test solutions because these had been prepared with water acidified with HCl.

Broderius (1973) explored more fully the toxicity of the silver-cyanide complex in solutions of varying salinity and the influence of chloride ion on the dissociation of the complex. The rapid dissociation of the $Ag(CN)_2^-$ ion in fresh water was found to be somewhat more extensive at equilibrium than that which was to be expected on the basis of calculations that could be made, using any one of the recently published values for the dissociation constant of the ion. As in the case of the nickelocyanide complex, the apparent dissociation constants for the $Ag(CN)_2^-$ ion computed from determined HCN concentrations in solutions of varying total cyanide content and pH, all at 20° C, varied considerably, perhaps largely because of differences in ionic strength of the solutions. Values computed from results of tests of solutions with total cyanide levels of 0.5 to 20 mg/l (pH 6.0-8.5) and 100 to 200 mg/l (pH 7.1-7.5) averaged 0.86×10^{-19} and 8.4×10^{-19}, respectively. The over-all average of the 14 computed values, pertaining mostly to total cyanide concentrations of about 10 mg/l (5-20 mg/l), was determined to be 1.94 x 10^{-19} and by some 1 to 3 orders of magnitude greater than the recently published values for the constant.

Even when a value as high as 2 x 10^{-19} is used as the dissociation constant for the $Ag(CN)_2^-$ ion in calculating degrees of dissociation in very dilute solutions, complexation of cyanide with silver can be seen to be quite effective in preventing the occurrence in cyanide-contaminated fresh waters of sublethal levels of free cyanide believed to be harmful to fish. An example of such a calculation, demonstrating the relative ineffectiveness of complexation with nickel, already has been presented. My similar calculation, in which 2 x 10^{-19} was taken to be the correct value for K_D, showed that, when the molecular HCN or free cyanide level in a solution in which cyanide is complexed with silver is only 0.005 mg/l (2 x 10^{-7} M), most of the total cyanide present (more than 80 percent) must still be bound in the complex ions even at a pH as low as 7.0. At pH 8.0, the dissociation is shown to be very slight, a concentration of the complex (5 x 10^{-5} M) equivalent to about 2.6 mg/l of cyanide as CN apparently being required to produce a molecular HCN level of 0.005 mg/l.

Fresh water and sea water diluted to several different levels of chlorinity (concentration of chloride ion, Cl^-) were used by Broderius (1973) as diluents in preparing test solutions containing 10 mg/l of cyanide (as CN) complexed with silver and having pH values near 7.7. The molecular HCN concentrations in these solutions were found to increase linearly from less than 0.01 to about 0.24 mg/l with increase of the chlorinity from nearly 0 to about 8.6 parts per thousand (ppt), the chlorinity of a 50 percent dilution of the sea water used. With further increase of the chlorinity to about 17.2 ppt (the chlorinity of the sea water), the HCN concentration did not continue to increase, remaining at the level of about 0.24 mg/l. The median survival time of euryhaline threespine sticklebacks in a solution prepared with water that was 10 percent sea water (chlorinity less than 2 ppt) was 786 minutes and nearly the same as it was in the fresh-water solution (770 minutes), although the HCN content had increased to about 0.05 mg/l. With further increase of the chlorinity to that of the full-strength sea water, however, the median survival time declined progressively in a curvilinear fashion; it was 140 minutes at the chlorinity of the full-strength sea water and 260 minutes at the chlorinity one-half as great. These results show that the toxicity of the solutions of low chlorinity was independent of the very low concentrations of molecular HCN and silver ion, having been determined by the virtually constant concentration of the moderately toxic $Ag(CN)_2^-$ ion. The products of the dissociation of the complex must have been largely or entirely responsible for the speedier lethal action of the more saline solutions. The high HCN concentrations in the solutions prepared with the more saline waters (50 to 100 percent but not 25 percent sea water) apparently could have alone caused the death of the fish in these solutions. The median survival times of the sticklebacks in these saline solutions were approximately those that were to be expected on the basis of results of some tests of comparable NaCN solutions with the same chlorinities and not much different molecular HCN levels. The latter tests, the results of which already have been reported, revealed an influence of water salinity on the toxicity of free cyanide or HCN quite similar to its effect on the toxicity of the solutions of cyanide complexed with silver that had a high and uniform determined HCN content. The toxicity of the $Ag(CN)_2^-$ ion may increase with increase of water salinity, as did the toxicity of free cyanide, but such an effect is not clearly indicated.

The 24-hour median tolerance limits of the silver-cyanide complex for sticklebacks in fresh water and sea water of about 17 ppt salinity were found by Broderius to be approximately 6 and 3 mg/l, respectively, at pH 7.7 and 7.9 and at a temperature of 20° C. The concentrations of molecular HCN in the test solutions prepared with sea water were below

0.1 mg/l, but this toxicant may have been at least partly responsible for the acute toxicity of the solutions. In the fresh-water solutions (pH 7.7), the HCN concentrations were well below 0.01 mg/l and hardly could have contributed materially to the measured, acute toxicity of the solutions.

Bluegills proved more resistant to the $Ag(CN)_2^-$ ion in fresh water than were the sticklebacks. The 24-hour median tolerance limit for the bluegills at pH 7.5-8.5 and 20° C evidently was not far above 10 mg/l as CN, the median survival times (for 10 fish) having been 29 and 31 hours at this concentration of the complex in solutions with pH 7.5 and 8.5 and determined HCN concentrations of 0.0123 and 0.0032 mg/l, respectively. Perhaps because of difference in source or physiological condition of the test animals, these results are not in very good agreement, however, with those of Doudoroff, Leduc, and Schneider (1966), who recorded a median survival time of bluegills of only about 14 hours in a solution containing 10 mg/l of cyanide (as CN) complexed with silver, at pH 7.5 and an HCN concentration of about 0.02 mg/l (perhaps less). On the other hand, Broderius' results fully support the view that the products of dissociation of the silver-cyanide complex cannot contribute materially to the acute toxicity of solutions prepared with fresh water and having pH values near or above 7.5. It can be seen that the median survival time of the bluegills in the presence of 10 mg/l of the complex as CN decreased very little with a decrease of pH from 8.5 to 7.5 and a nearly fourfold increase of HCN concentration from the extremely low level of 0.0032 mg/l, which obviously could have had no appreciable effect on the toxicity of the solution. Broderius also reported that the median survival time of bluegills at a concentration of the silver-cyanide complex of 7 mg/l as CN, pH 8.0, and a molecular HCN level of 0.0068 mg/l was 87 hours. A 96-hour median tolerance limit of the $Ag(CN)_2^-$ ion somewhat below 7 mg/l as CN is indicated.

Broderius noted that the reaction of silver ion with chloride ion present in sufficiently high concentration yields complex silver-chloride ions, $AgCl_2^-$ and $AgCl_3^{-2}$, as well as very slightly soluble silver chloride, AgCl, with which chloride ions combine to form the complexes before any AgCl can precipitate. By calculation, he found that the $Ag(CN)_2^-$ ion must be the predominant silver complex species in a solution with a CN/Ag mole ratio of 2.0 and containing chloride ion, which competes with the cyanide ion for the silver ion. As the Cl^- ion concentration increases from a low level, the dissociation of the $Ag(CN)_2^-$ complex ion with liberation of CN^- ion also increases because of the intensified competition by the Cl^- ion for the Ag^+ ion, but the extent to which the cyanide complex can be thus decomposed must be quite limited, the equilibrium level of free cyanide approaching an asymptote when most of this complex is still intact. The observation that, in the solutions prepared with diluted sea water and containing 10 mg/l of cyanide (as CN) initially complexed with silver, the HCN concentration did not increase appreciably beyond the level of about 0.24 mg/l with increase of chlorinity beyond 8.6 ppt at a constant pH of 7.7 is in agreement with that conclusion. It is noteworthy, however, that when the chlorinity of a solution containing only 1 mg/l of cyanide complexed with silver was increased by the addition of sodium chloride, NaCl, the HCN concentration continued to increase markedly, at pH 7.7, with increase of chlorinity beyond 0.25 M, or 8.9 ppt, the chlorinity of about a 50 percent dilution of sea water. It attained a level of 0.052 mg/l at a chlorinity of 20.8 ppt, but was only 0.028 mg/l at the chlorinity of 8.9 ppt. The relatively low concentration of the cyanide complex in the NaCl solutions may be the main reason why a maximum HCN level was not nearly attained at the lower chlorinity; similar tests with higher concentrations of the complex and with as much NaCl added were not performed by Broderius.

Evidence of penetration of the silver-cyanide complex ion into the bodies of bluegills has been presented by Broderius, who studied the rates of accumulation of silver in various tissues of fish exposed for brief periods to solutions containing the complex or only $AgNO_3$, all with a pH of 8.0. Although the silver ion was found to penetrate quite readily into the bodies of the fish, its concentrations in the tested solutions in which the silver was complexed with cyanide were found by calculation to have been too low for the entry of this ion to have been responsible for much of the observed accumulation of silver in the internal tissues of fish exposed to these solutions. When the fish were exposed to $AgNO_3$ solutions, silver tended to accumulate most markedly in gill tissues, whereas fish exposed to solutions in which the silver was complexed with cyanide accumulated little silver in the gill tissues, and accumulation in their internal organs and blood was more pronounced than accumulation in the gills. The silver-cyanide complex ion evidently passes through the gills and into internal organs of fish more readily than does the nickelocyanide complex, but not quite as readily as does the cuprocyanide complex ion $Cu(CN)_2^-$ (to be considered presently), whose more rapid penetration may be the reason for its greater toxicity.

Silver ion is known to be extremely toxic to fish (one of the most toxic ions), and its concentration, in mg/l, must be about twice the HCN concentration in a fresh-water solution in which the CN/Ag mole ratio is 2.0, if there is no withdrawal of either silver ion or cyanide ion from the system except by the complexation of silver with cyanide. It is quite possible, therefore, that at very low concentrations of the silver-cyanide complex and low levels of pH at which the concentration of neither the complex ion nor of speedily acting HCN is high enough to be soon fatal to fish, the toxicity of the free silver ion is often predominant. The silver ion under these circumstances could well be the toxicant principally or entirely responsible for slow death of fish, or cause chronic, sublethal injury to them more serious than the effects of the low concentrations of HCN and of the $Ag(CN)_2^-$ ion. The chronic toxicity of metallocyanide complexes has not yet been investigated.

COPPER-CYANIDE COMPLEXES

In early experiments on the toxicity of the cuprocyanide complexes reported by Stumm, Woker, and Fischer (1954), Lipschuetz and Copper (1955), and Bucksteeg and Thiele (1957), the CN/Cu mole ratio ranged from 3.0 to 4.0. The amounts of the cyanide group in the test solutions thus greatly exceeded the minimum amounts necessary for combination with all of the copper(I) present to form the dicyanocuprate(I) complex ion, $Cu(CN)_2^-$. Marked reduction of the toxicity of cyanide upon its complexation with copper(I) was demonstrated, but all of the tested combinations of cyanide and copper proved quite toxic, even though the solutions tested by Stumm, Woker, and Fischer, with a reported CN/Cu ratio of 3.0, were very alkaline (pH 9.0-9.4).

The most detailed of the early studies apparently was that of Lipschuetz and Cooper (1955), whose test animal was the western blacknose dace, Rhinichthys atratulus meleagris. They found that the 24-hour median tolerance limits for this fish of KCN alone and of KCN combined with cuprous cyanide, CuCN, to produce cuprocyanide solutions with CN/Cu mole ratios of 4.0, 3.75, and 3.0 were, respectively, about 0.22, 0.38, 0.47, and 0.71 mg/l as CN. It is evident that the toxicity of solutions of equal total cyanide content decreased considerably with increase of the amount of copper present, which must have resulted in decrease of the concentration of free cyanide. The pH of the test solutions ranged from 7.6 to 8.0, and the temperatures from 20 to 22° C. The test solutions were not aged to ensure attainment of equilibria, and, judging by the data of Broderius (1973) on the rates of formation of cuprocyanide complexes, one has good reason to doubt that equilibria had been nearly attained. Other investigators presumably have dissolved or have diluted already formed cuprocyanide complexes in preparing test solutions, and the dissociation of these complexes, like their formation, can be slow. Incomplete dissociation, as well as unduly high levels of pH or misidentification of compounds tested, may have led to some underestimation of the toxicity of cuprocyanides with high CN/Cu mole ratios.

Doudoroff (1956) and Doudoroff, Leduc, and Schneider (1966) tested dilutions of relatively concentrated solutions of somewhat uncertain composition, which were prepared by adding a solution of cupric sulfate, $CuSO_4$, to an NaCN solution until a precipitate (which was not highly persistent, dissolving eventually) just began to appear. The mole ratio of initially introduced cyanide to copper was about 3.0. However, as Doudoroff, Leduc, and Schneider have pointed out, some of the cyanide initially present must have been lost through oxidation (formation of cyanogen, which then undergoes hydrolysis, yielding equivalent amounts of cyanate ion and regenerated free cyanide) as copper(II) was reduced to copper(I). The final CN/Cu mole ratio thus was believed to have been some value well above 2.0 but not exceeding 2.5. Even in slightly alkaline test solutions (pH 7.2-7.9), much free cyanide was present, and all of the observed toxicity to bluegills of solutions tested by Doudoroff, Leduc, and Schneider could be attributed to the determined HCN content. Still, pronounced detoxification of both free cyanide and cupric ion was shown to result from their reaction when they are combined. Doudoroff (1956) found the 24-hour median tolerance limit of the mixture for the fathead minnow in a soft water of nearly neutral reaction to be equivalent to about 2.2 mg/l of cyanide (as CN) initially present (total cyanide introduced), or 1.8 mg/l of copper. The corresponding values for NaCN alone and $CuSO_4$ alone were 0.25 mg/l as CN and less than 0.1 mg/l as Cu, respectively, under static bioassay conditions. The detoxification of the combined toxicants was observed both when a fairly concentrated solution of NaCN (about 940 mg/l as CN) was mixed with a fairly concentrated solution of $CuSO_4$ (1000 mg/l as Cu) and when only 0.8 mg of cupric ion per liter was added to an NaCN solution with a cyanide content of only 1.0 mg/l as CN. However, solutions prepared in the last-mentioned manner about 1 hour before the introduction of test animals proved somewhat toxic, killing about half of the fish (55%) in 96 hours, whereas a comparable dilution of the more concentrated mixture killed no fish in 96 hours, and the 96-hour median tolerance limit of this mixture was found to be about 1.5 mg/l as CN initially present, or 1.2 mg/l as Cu. Addition to the concentrated mixture of enough $CuSO_4$ or $NiSO_4$ to produce abundant precipitates (i.e., pronounced turbidity) did not markedly reduce its toxicity in the moderately soft dilution water.

When diluted with extremely soft, nearly neutral or faintly acid water, and especially in pure, distilled water (at pH near 6.0), the above-described, initially concentrated mixture proved decidedly more toxic than it was in the water that was not quite so soft and that was slightly alkaline (pH about 7.5) when it was continuously or had been recently well aerated. Prolonged aeration with compressed air, before the introduction of fish only, or continued also thereafter, of the test solutions prepared with the very soft water increased their toxicity markedly. Aging of such a solution in stoppered bottles had a similar but much less pronounced effect. The behavior and appearance of fish affected or dying in the solutions prepared with very soft or distilled waters were suggestive of poisoning with copper, rather than cyanide. Doudoroff surmised that the toxicity of these solutions probably was due to the presence of copper ions deriving from dissociation and gradual decomposition of relatively harmless and stable copper-cyanide complex ions, although it could not be shown that increased toxicity of the complex ions in the very soft waters was not a factor. Copper is known to be extremely toxic to fish in distilled and very soft waters and presumably was liberated increasingly from cuprocyanide complex ions as free cyanide (volatile HCN), also deriving from their dissociation, was removed by prolonged aeration of the neutral solutions. Aeration for 4 days (before the fish were introduced) of test solutions containing 0.5 mg/l of copper combined with cyanide that were prepared with the only moderately soft water (total alkalinity 17.0 mg/l as $CaCO_3$; total hardness 25 mg/l as $CaCO_3$; pH during aeration 7.5) did not render these solutions demonstrably toxic to the minnows. Whether the aeration was continued or not, the solutions did not prove fatal to the fish within 10 days.

Broderius (1973) studied the toxicity to bluegills of cuprocyanide solutions with CN/Cu mole ratios of 2.0, 2.5, and 3.0, prepared by dissolving CuCN in very dilute solutions of NaCN in buffered well water, which were confined in carboys and agitated with magnetic stirrers. Solutions of varying total cyanide content and having different, widely ranging pH values (6.51-8.97) were tested, the concentration of molecular HCN in each one was determined, and the concentrations of the $Cu(CN)_2^-$ and $Cu(CN)_3^{-2}$ complex ions at assumed equilibrium were calculated. It is noteworthy, however, that equilibria previously had been found not to have been attained, for reasons not known, in most of the examined solutions with CN/Cu mole ratios of 2.5 or 3.0 even after their prolonged storage. In solutions like those used in the toxicity tests with a CN/Cu mole ratio of 2.0, on the other hand, equilibria (indicated by constancy of HCN levels) were found to be attained fairly rapidly, within 2 or 3 days; therefore, the results of toxicity tests of the solutions with this mole ratio, which were used in most of the tests, are deemed the most meaningful ones. In all of the tested solutions except two very dilute and acid ones (total CN 0.2 mg/l; pH 6.52-6.57) with CN/Cu mole ratio of 3.0, in which free cyanide was predominant, the cyanide group was seen to occur very predominantly in $Cu(CN)_2^-$ ions, but the $Cu(CN)_3^{-2}$ ion apparently can be predominant at equilibrium in other, similar solutions with a CN/Cu ratio of 3.0 or even less. Concentrations of the $Cu(CN)_4^{-3}$ ion were believed to have been in all cases negligible, and in solutions with CN/Cu mole ratio of 2.0, those of the $Cu(CN)_3^{-2}$

ion apparently were and would have remained without exception very low in comparison with $Cu(CN)_2^-$ ion concentrations, though some $Cu(CN)_3^{-2}$ ion and some remaining CuCN must have been always present. Unreliability of presently accepted stepwise dissociation constants pertaining to cuprocyanide complex ions was suggested by Broderius' chemical data, but the K_D value for the $Cu(CN)_2^-$ ion computed by him from equilibrium HCN levels in his solutions, about 4×10^{-24}, agreed well with previously published values.

The median survival times of bluegills in the few tested solutions with CN/Cu mole ratios of 2.5 and 3.0 corresponded closely with those of bluegills exposed to NaCN solutions (at pH 7.5) having molecular HCN concentrations nearly equal to the determined HCN concentrations in these cuprocyanide solutions. The acute toxicity of these solutions thus was clearly attributable to their high molecular HCN content, 0.134-0.269 mg/l as HCN; HCN then proved lethal to the bluegills in about 11 hours (median survival time) at a level as low as 0.130 mg/l in a solution of NaCN having an initial concentration of 0.15 mg/l as CN (amount added). The cuprocyanide solutions had total cyanide concentrations of only 0.2 to 1.0 mg/l as CN and pH values of 6.5 to 7.5, and the molecular HCN levels were about one-fifth to nearly four-fifths of the total cyanide concentrations.

When the CN/Cu mole ratio was 2.0 and the total cyanide concentrations (5.0 to 50 mg/l as CN) were more than 20 to 5000 times the molecular HCN concentrations, the median survival times of the bluegills in the cuprocyanide solutions were invariably less than those recorded or estimated for NaCN solutions with the same HCN levels. In solutions with determined HCN concentrations less than 0.025 mg/l and total cyanide concentrations of 5, 15, 25, and 50 mg/l, the median survival times were, or averaged, 2520, 632, 354, and 199 minutes, respectively. As the pH of the solutions of each total cyanide content was reduced and their molecular HCN content was thus increased, their toxicity increased only slightly, or remained nearly constant, or even declined somewhat (when the total cyanide concentration was 25 or 50 mg/l) for some undetermined reason, until a molecular HCN level of about 0.04 to 0.07 mg/l was attained. With further decrease of pH and increase of molecular HCN, the toxicity increased more or less sharply, evidently becoming dependent increasingly on the concentration of HCN and less on the concentration of cuprocyanide complex ions or total cyanide. These sharp increases of toxicity occurred when the pH was reduced to levels below a value of about 7.6-8.0 at all the total cyanide concentrations tested. Even in the solutions of lower pH and relatively high HCN content, the fish tended to die sooner than those exposed to the same HCN levels in NaCN solutions. The undissociated cuprocyanide ions and perhaps also the copper ions present evidently contributed to the toxicity of these solutions. It should be noted, however, that the total cyanide concentrations in the tested solutions with CN/Cu mole ratio of 2.0 were not very low; had solutions been tested with much lower total cyanide levels (i.e., 1.0 mg/l or less) and pH low enough to cause extensive dissociation of the $Cu(CN)_2^-$ ions and thus to render the solutions acutely toxic, almost all of their toxicity probably would have been found to be attributable to their HCN content.

There can be little doubt that the high toxicity of tested solutions with very low HCN levels (sometimes less than 0.01 mg/l) was due entirely or almost entirely to toxic action of the cuprocyanide complex ions, the levels of free cyanide and copper in these solutions having been apparently much too low to have been rapidly effective. The 48-hour median tolerance limit of the $Cu(CN)_2^-$ ion for bluegills in slightly alkaline solutions in which this complex ion is the only toxicant present in considerable amounts was estimated by Broderius to be about 9 mg/l as $Cu(CN)_2^-$, or 4 mg/l as CN, at 20° C. By determination of amounts of copper accumulated in various tissues of bluegills exposed to highly toxic cuprocyanide solutions with very low (presumably negligible) levels of free cyanide and copper, the $Cu(CN)_2^-$ complex ion's ability to enter the bodies of fish quite rapidly was revealed. The penetration rate was estimated to be nearly four times that of the silver-cyanide complex, and this may be the reason why the cuprocyanide ion proved more toxic than the silver-cyanide complex ion in like toxicity tests. In the absence of rapidly fatal concentrations of molecular HCN, the cuprocyanide complex was found to produce signs of intoxication strongly resembling signs of poisoning with free copper and other heavy metals, as did also the silver-cyanide complex. Much coagulated mucus was observed on the gills and body surfaces of the affected fish.

IRON-CYANIDE COMPLEXES

Data in early literature indicating little toxicity to fish of the iron-cyanide complexes have been contradicted by some more recently published experimental results but are in agreement with other, even later findings. Oshima (1931) has reported that 2.5×10^{-5} M solutions of potassium ferrocyanide, $K_4Fe(CN)_6$, and potassium ferricyanide, $K_3Fe(CN)_6$, killed young eels, Anguilla japonica, in about 5 to 6 hours, or almost as rapidly as did a 1×10^{-5} M solution of KCN (0.26 mg/l free cyanide as CN). The total cyanide content of a 2.5×10^{-5} M solution of $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$ is only 3.9 mg/l as CN. On the other hand, Burdick and Lipschuetz (1950) stated that 4,000 mg/l of $K_4Fe(CN)_6$ (about 1,700 mg/l as CN), but not 2,000 mg/l, proved fatal to fish (cyprinids) within 24 or 48 hours when the solutions were kept in the laboratory in diffused light or in the dark. However, only 2 mg/l (0.85 mg/l as CN) killed blacknose dace, Rhinichthys atratulus, creek chubs, Semotilus atromaculatus, and silvery minnows, Hybognathus regius, in 1 to 1.5 hours when the solutions were tested in direct sunlight after their preliminary exposure to the light for 0.5 to 1.5 hours. Under the same conditions, 2 mg/l of $K_3Fe(CN)_6$ (0.95 mg/l as CN) killed creek chubs and emerald shiners, Notropis atherinoides, in 13-38 minutes. Bucksteeg (1961) found that a total cyanide concentration of nearly 300 mg/l as CN could be tolerated for 24 hours by rainbow trout in a fresh solution of $K_4Fe(CN)_6$ kept in the dark, but less than 30 mg/l total CN proved fatal within 24 hours when the solution was exposed to diffused daylight, and only 2 mg/l caused death within 8 hours when the solution was illuminated with direct sunlight. The susceptibility of the iron-cyanide complexes to rapid photolysis (photodecomposition) producing free cyanide must be the main reason for the large differences of toxicity test results reported in the literature. The extent of this decomposition in experimental solutions obviously can vary greatly with the nature or intensity of illumination of the solutions before and during the tests. Considerable toxicity of the iron-cyanide complex ions, which slowly dissociate to some extent even in the dark, has not been demonstrated.

Broderius (1973) found that bluegills lived for more than 48 hours in a distilled water solution of $K_3Fe(CN)_6$ that had been kept in the dark in a closed vessel for a long time (309 days) and had a pH of 7.1, a total cyanide content of 500 mg/l as CN, and a determined molecular HCN concentration of only 0.067 mg/l, well below the minimum acutely toxic level. The median survival time of bluegills in a $K_4Fe(CN)_6$ solution with the same total cyanide content (500 mg/l as CN) and the same pH (7.1), and also thoroughly aged for about 10 months to ensure equilibrium, was only 145 minutes. However, the determined molecular HCN content of this solution was 0.267 mg/l, and the recorded median survival time of the fish was approximately that which was to be expected at the determined HCN concentration on the basis of tests of NaCN solutions. It is quite apparent that the undissociated ferrocyanide ion did not contribute materially to the lethality of the ferrocyanide solution (which may have been deficient in dissolved oxygen), although the concentration of complexed cyanide was almost 2,000 times that of free cyanide. Ferrocyanide solutions kept in the dark clearly can become much more toxic to fish than comparable ferricyanide solutions with the same total cyanide content, because of greater instability of the former complex.

Broderius also observed that the molecular HCN concentration in a dilute $K_4Fe(CN)_6$ solution with a total cyanide content of only 5 mg/l and kept in the dark increased progressively for about 2 months to nearly 0.06 mg/l. It then declined sharply to a level near 0.01 mg/l and was nearly constant thereafter, remaining at that level to the end of the 10-month experiment. This final concentration was found to be very nearly the same as the equilibrium concentration in a $K_3Fe(CN)_6$ solution with a total cyanide content of 5 mg/l. The pH of both solutions was 6.8 and the temperature 20° C. Broderius concluded that the decline of the HCN concentration in the ferrocyanide solution had been due to oxidation of the iron in the presence of molecular oxygen ($O_2 + 4Fe^{+2} + 4H^{+} \longrightarrow 4Fe^{+3} + 2H_2O$), the oxidation resulting in complete conversion of the ferrocyanide to ferricyanide. This explanation was fully supported by the results of comparative studies of ultraviolet absorption spectra of the dilute ferrocyanide and ferricyanide solutions stored in the dark for different periods of time. It was supported also by observed increases of the pH of the ferrocyanide solution requiring repeated additions of sulphuric acid for maintenance of a nearly constant pH. The ultraviolet absorption spectrum of a more concentrated ferrocyanide solution with a total cyanide content of 500 mg/l changed only slightly with time, presumably because of the limited amount of available oxygen, and the spectra of both dilute and relatively concentrated ferricyanide solutions remained unchanged.

Broderius noted that the molecular HCN concentrations and the toxicity of his thoroughly aged ferrocyanide and ferricyanide solutions were not nearly as great as those predictable through computation of the equilibrium HCN levels based on currently accepted values of the cumulative dissociation constants for the complexes. His own "best estimates" of these constants, derived by computation from the measured equilibrium levels of molecular HCN, were about 10^{-47} for the ferrocyanide ion and 10^{-52} for the ferricyanide ion at 20° C. These apparent or formal K_D values are smaller by about 11 and 9 orders of magnitude, respectively, than K_D values recently reported in the chemical literature. The value 10^{-47} for the ferrocyanide ion was said to be perhaps slightly too low an estimate, because of some probable oxidation of the iron in the fairly concentrated ferrocyanide solution (with 500 mg/l total CN). The equilibrium HCN concentration in this solution was used in the computation without any correction for the probable conversion of some of the ferrocyanide (a small portion) to ferricyanide. A need for reconciliation of the earlier published dissociation constants in question with the observations of Broderius is evident. Some of the possible reasons for the apparent discrepancy between them already have been noted. The influence of pH on ferrocyanide or ferricyanide concentrations required to produce a given level of HCN or toxicity, which can be reasonably expected to be even more pronounced than the corresponding effect observed in experiments with the nickelocyanide complex, was not evaluated by Broderius. Such an additional study, and also a study of dissociation rates and equilibrium HCN concentrations in dilute ferrocyanide solutions devoid of free oxygen, could be quite instructive.

From the information presented above, one can conclude that, were it not for the phenomenon of photolysis, surface waters receiving moderate amounts of the ferrocyanide and ferricyanide complexes probably would never be rendered thereby acutely toxic to fish. Some of the HCN produced by dissociation of the complexes would be constantly escaping to the atmosphere or be otherwise lost, and the ferrocyanide complex would be gradually converted into the more stable ferricyanide complex. Therefore, and because of the low rate of dissociation of the complexes, the low equilibrium levels of HCN would never be attained, and the attainment of acutely toxic HCN levels even in waters contaminated with the ferrocyanide ion would be most improbable. The occurrence of harmful, sublethal levels of HCN deriving from the complexes would still be possible, for Broderius (1973) has observed HCN concentrations exceeding 0.01 mg/l even in very recently prepared solutions of both $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ with a total cyanide concentration of only 5 mg/l. However, it is mainly because of the susceptibility of the two complexes to photodecomposition that their disposal in surface waters must be regarded as potentially harmful or dangerous to fish and other aquatic life. The computation of equilibrium levels of HCN in solutions not exposed to light can be of very little or no practical importance.

Burdick and Lipschuetz (1950) determined free cyanide levels in ferrocyanide and ferricyanide solutions by a colorimetric method that apparently is sufficiently reliable when only the stable iron-cyanide complexes are present. They found that a free cyanide concentration of 0.3 mg/l as CN, which previous tests had shown to produce a 50 percent mortality of blacknose dace, Rhinichthys atratulus, and creek chubs, Semotilus atromaculatus, in 5.25 to 7.5 hours, could be attained in solutions of $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ with a total cyanide content little more than twice as great by exposing them to direct sunlight. The concentrations of the two salts required for producing this level of free cyanide in solutions exposed to direct sunlight in open vessels were reported to be 1.45 mg/l $K_4Fe(CN)_6$ and 1.34 mg/l $K_3Fe(CN)_6$, i.e., about 0.61-0.64 mg/l as CN. The authors noted that lower concentrations perhaps would have been sufficient had the experiments been performed in summer. Water quality differences were reported to have had a considerable influence on the liberation of cyanide (i.e., on amounts measured as free cyanide) in experiments that were alike in other respects.

In reporting concentration values in their paper, Burdick and Lipschuetz did not distinguish properly between initial ferrocyanide or ferricyanide ion concentrations and initial concentrations by weight of the entire salt molecules, including the potassium, whose contribution to the weight of the molecules (unlike that of the cyanide groups) changes with the valence or oxidation state of the iron. Thus, some values reported or referred to as ferrocyanide or ferricyanide concentrations (e.g., in Tables 1 and 2) are elsewhere shown to be concentrations of $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$ (e.g., in Figure 4). Although comparison of solutions of the two salts having the same molarity and total cyanide content would appear to be most appropriate and instructive, after careful examination of the paper I concluded that all the concentration values given there (other than free cyanide concentrations) must be concentrations by weight of the entire molecules, including potassium. Lipschuetz (personal communication) has informed me that this conclusion is in agreement with his understanding and recollection. The "cyanide ion" (CN^-) concentrations reported are obviously levels of all free cyanide, including HCN.

Burdick and Lipschuetz stated that a part of the photodecomposition of the iron-cyanide complexes is reversible in the dark or in weaker light, but no definite evidence of partial reversibility of the reaction was presented by them. Recombination of free cyanide with other decomposition products and permanent loss of the cyanide after its liberation in their tests were not readily distinguishable. Doudoroff (1956) found that very little if any combination of free cyanide with ferrous ion to form a complex occurred when only 0.33 mg/l of free cyanide as CN and 0.13 mg/l of ferrous ion were introduced into a soft, slightly alkaline water by the addition of solutions first of NaCN and then of $FeSO_4$. A colloidal precipitate, supposed to have been ferric hydroxide, soon rendered the water brown, and, after standing for 3 hours, the water remained highly toxic to fathead minnows, killing all within 24 hours. The ferrocyanide complex evidently did not form readily also in similar, very dilute solutions prepared by adding first NaCN and then $FeSO_4$ to distilled water, although much complexation of cyanide with iron obviously did occur when much more concentrated solutions of the two salts in distilled water were combined.

In the British Water Pollution Research Laboratory (Great Britain, Department of the Environment, 1972), the toxicity to rainbow trout fry of a saline solution containing 2.0 mg/l of $K_4Fe(CN)_6$ and exposed to direct sunlight in summer was evaluated by constant-flow bioassay at a low temperature of 5° C. The reason for performing these tests was that $K_4Fe(CN)_6$ has been added, to prevent caking, to salt used for de-icing road surfaces in winter. The test solutions were prepared with hard water to which NaCl was added in the amount of 1.54 percent, and they were irradiated for about 16 hours before the toxicity tests; the intensity of irradiation was reported to have been about 90,000 lux. Tests of equally saline solutions of KCN also were performed for comparative purposes. The irradiated ferrocyanide solutions proved somewhat less toxic than KCN solutions with the same total cyanide content, the test results suggesting that only about one-half of the cyanide originally bound in the complex was present as free cyanide, and thus being in good agreement with the findings of Burdick and Lipschuetz. The "apparent" 72-hour median lethal concentration of $K_4Fe(CN)_6$ in the irradiated, cold, saline solution was found to be about 0.17 mg/l. This value is equivalent to 0.07 mg/l of cyanide as CN, which is approximately the 24-hour median lethal concentration of free cyanide as CN indicated by the results of the tests with KCN solutions.

Myers and Iezzi (1950) have reported results of some experiments in which dilute test solutions of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in a hard water were irradiated with ultraviolet lamps for not more than 3 hours and tested for toxicity to bluegills and yellow perch, *Perca flavescens*. The 24-hour median tolerance limits of both salts in the irradiated solutions were found to be usually near 0.5 mg/l and always less than 0.7 mg/l. There is no indication that the reported values are concentrations of cyanide as CN and not of the entire, double salts. Inasmuch as 0.5 mg/l of $K_3Fe(CN)_6$ and 0.5 mg/l of $K_4Fe(CN)_6$ are equivalent to 0.24 and 0.21 mg/l of cyanide as CN, respectively, and the 24-hour median tolerance limit of KCN was reported by Myers and Iezzi to have been about 0.375 mg/l, or 0.15 mg/l as CN, liberation of much more than half of the complexed cyanide in the irradiated solutions is indicated. If some recombination of the liberated cyanide with other photodecomposition products occurred after the brief irradiation of the test solutions, the decomposition could have been almost total, but such recombination is not indicated by the reported data. When tests were performed in "ordinary laboratory light", bluegills showed no distress after exposure for 75 hours to 1000 mg/l solutions of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, and 50 mg/l solutions had no effect on yellow perch in 24-hour tests.

Waters receiving cyanide-bearing wastes usually are not perfectly clear and very shallow, and often are quite turbid and deep. Because the penetration of sunlight, and especially that of ultraviolet light, is limited, photolysis of the iron-cyanide complexes in most of the receiving waters exposed to sunlight probably is not nearly as rapid as that observed in aquarium tests. Free cyanide produced by the photolysis is continuously lost to the atmosphere or otherwise eliminated. Therefore, concentrations of free cyanide lethal to fish may not be often attained even in surface waters receiving large amounts of the iron-cyanide complexes. Fish mortalities apparently attributable to photolysis of these complexes in streams receiving industrial effluents that were known to contain the pollutants have been reported, however, by Burdick and Lipschuetz (1950) and by Myers and Iezzi (1950). The source of the waste involved was not stated by the former authors, who reported a fish mortality extending over 12 miles (7.5 kilometers) of a stream in the State of New York but no details of this occurrence. The fish mortalities reported by Myers and Iezzi occurred in the summer of 1949 in Tulpehocken

Creek, Lebanon County, Pennsylvania, and were believed to have been probably due to pollution with wastes from a ferromanganese blast furnace escaping from a leaking waste-treatment lagoon. No free cyanide was found in the wastewater and in stream water samples taken at locations where the death of fish had previously been observed, but "appreciable amounts of ferro- and ferricyanide" were said to have been found in the seepage from the waste-treatment lagoon and in a heavy silt deposit on the stream bottom. Cyanide was found in the tissues of dead fish in amounts believed to have been sufficient to have caused death, and local residents had reported that all the fish mortalities occurred at midday on bright, sunny days.

It may be helpful to note here that no increase of the toxicity to fathead minnows of solutions containing the nickelocyanide and cuprocyanide complexes upon prolonged exposure of the solutions to direct sunlight was observed by Doudoroff (1956), and none was to be expected.

Hiatt, Naughton, and Matthews (1953b) reported that the irritant activity of $K_3Fe(CN)_6$ on the marine fish Kuhlia sandvicensis was slight at a concentration of 1.0 mg/l and moderate at a concentration of 10 mg/l. Corresponding effects of KCN were reported to have been observed at concentrations of 0.1 and 1.0 mg/l, respectively. The significance of these observations is not clear, and their reliability can be reasonably questioned, in view of the low toxicity of the ferricyanide ion to freshwater fishes. It is not evident that experiments with each of the very numerous chemicals tested were repeated often and carefully enough to preclude error.

SECTION VI

TOXICITY OF OTHER, RELATED COMPOUNDS

NITRILES

The nitriles, or organic cyanides, vary widely in their toxicity to fish. Acetaldehyde cyanohydrin, $CH_3CH(OH)CN$, also known as lactonitrile, but more appropriately considered as a cyanohydrin because of its distinctive structure and behavior of toxicological importance, undergoes rapid decomposition or hydrolysis in aqueous solutions, yielding CN^- ion and HCN. The toxicity of a solution of this compound consequently differs little if any from that of a solution of KCN or NaCN with the same content of the cyanide (CN) group. Daugherty and Garrett (1951) found the 24-hour median tolerance limit of lactonitrile for the marine pin perch, Lagodon rhomboides, in unrenewed solutions in sea water at variable temperatures of 13.7 to 20.4° C to be about 0.22 mg/l (0.08 mg/l as CN). Renn (1955) reported 0.51 mg/l (0.19 mg/l as CN) in fresh water to have proved fatal to some bluegills, white crappie, Pomoxis annularis, and golden shiners, Notemigonus crysoleucas, in constant-flow tests at 25° C, as well as some static bioassays. The tests lasted for 24 hours or longer, but deaths of all three species occurred within 10 hours. Henderson, Pickering and Lemke (1961) reported both the 24-hour and the 96-hour median tolerance limits at 25° C to have been about 0.90 mg/l (0.33 mg/l as CN) for fathead minnows in both soft and hard waters and for bluegills in soft water, and 1.37 mg/l (0.50 mg/l as CN) for guppies in soft water, when test solutions were not renewed. When the test solutions were renewed continuously by constant-flow replacement, the 24-hour, 96-hour, 5-day, and 20-day median tolerance limits for fathead minnows in soft water were found to be 0.75, 0.71, 0.69, and 0.69 mg/l, respectively (0.27-0.25 mg/l as CN).

The 24-hour median tolerance limit of acrylonitrile, CH_2CHCN, for the pin perch was found by Daugherty and Garrett (1951) to be about 25 mg/l (12 mg/l as CN), again in tests of unrenewed solutions in sea water at variable temperatures. Renn (1955) reported that the highest level that could be tolerated tor 24 hours by all of the white crappie used in his constant-flow tests was between 38 and 68 mg/l (12 and 25 mg/l as CN). Bandt (1953) observed that concentrations of 25 to 50 mg/l (12-25 mg/l as CN) in standing, aerated solutions eventually proved lethal to bleak, *Alburnus alburnus*, and roach, *Rutilus rutilus*, in tests at temperatures of 10.5 to 18° C and lasting not longer than 20 days. He concluded that the threshold concentration for prolonged exposure is in the neighborhood of 20-25 mg/l (10-12 mg/l as CN). When tests were performed without renewal of test solutions, Henderson, Pickering, and Lemke (1961) found the 24-hour median tolerance limits at 25° C for fathead minnows in hard water and for fathead minnows, bluegills, and guppies in soft water to be about 32.7, 34.3, 25.5, and 44.6 mg/l, respectively. The corresponding 96-hour values were found to be 14.3, 18.1, 11.8, and 33.5 mg/l. respectively (5.8-16.4 mg/l as CN). When fathead minnows were tested in continuously renewed solutions prepared with the soft water, their median tolerance limits for exposure periods of 1, 2, 4, 10, 15, 20, 25, and 30 days were found to be about 33.5, 17.8, 10.1, 6.9, 5.2, 4.2, 3.5, and 2.6 mg/l, respectively. These values were obtained by averaging the results of five like experiments at 25° C in which a total of 50 fish had been exposed to each of seven different concentrations of the poison. Inasmuch as the logarithms of the tolerance limits continued to decline almost rectilinearly with increase of exposure time from 10 to 30 days (also with increase of log time from 10 to 25 days, but seemingly not thereafter), it is evident that the lethal threshold concentration was not determined and must be well below 2.6 mg/l (1.3 mg/l as CN).

In the British Water Pollution Research Laboratory (Great Britain, Ministry of Technology, 1970), the median periods of survival of "trout" (probably rainbow trout) at some unspecified temperature, presumably in continuously renewed test solutions, were found to be about 2 and 100 days at acrylonitrile concentrations of 40 and 2.0 parts per million by volume, respectively. Since the density of acrylonitrile (g/ml) in almost exactly 0.8, these two concentrations correspond to 32 and 1.6 mg/l (about 16 and 0.8 mg/l as CN), respectively, according to my calculations. It was also reported that exposure of the trout to an acrylonitrile concentration of 60 parts per million by volume (48 mg/l) caused their delayed death within 10 days after their return to clean, running water. Jackson and Brown (1970) reported results of very similar if not the same experiments with rainbow trout, presenting somewhat different data in a graph from which nearly or exactly the same acrylonitrile concentrations (about 33 and 1.6 mg/l) corresponding to median survival periods of 2 and 100 days can be derived. In a table in which various toxicity data are summarized, however, the values given by the authors for the 48-hour median lethal concentration and the concentration that killed 50 percent of the trout in 100 days (in "hard water") are 70 mg/l and 2.2 or 2.8 mg/l, respectively. According to my calculations, these data do not agree with the data plotted in the graph; yet, I have found no other reason to believe that they represent results of a different experiment. A computational error may be involved, or some misunderstanding on my part. But whatever may be the correct value, it is evident that even the 100-day median lethal concentration is not nearly equal to the lethal threshold concentration, which must be considerably lower. The concentration corresponding to a median survival period of 10 days is about 6.4 mg/l, so the line representing the observed relation between the logarithms of toxicant concentration and of median survival time within the range of 2 to 100 days is only slightly curved. Thus, the median tolerance limit may continue to decrease materially (demonstrably) with increase of exposure time throughout the normal life span of the test animal, and it may decline to a value well below 1 mg/l within that time period. Jackson and Brown stated that deaths of rainbow trout exposed to an acrylonitrile solution and returned to clean water occurred some 5 to 10 days later. Temperatures again were not reported.

At the same laboratory, the toxicity to rainbow trout of malononitrile, $NCCH_2CN$, which is even more toxic than acrylonitrile and evidently also an accumulative poison, has been evaluated recently (Great Britain, Department of the Environment, 1973). Median survival periods of trout exposed to concentrations of 32, 5, and 0.5 mg/l were found to be about 5 hours, 32 hours, and 3 days, respectively. The curve relating survival time to concentration of the poison is unusual, and it suggests that the lowest concentration tested, 0.5 mg/l (0.39 mg/l as CN), is again much above the lethal threshold concentration, which may be exceedingly low, perhaps as low as that of free cyanide or even lower.

Henderson, Pickering, and Lemke (1961) tested four other nitriles and found all of them to be less toxic, and most of them very much less toxic, than acrylonitrile. Only 96-hour static toxicity bioassays of these compounds were performed, without renewal of the test solutions, and the 96-hour median tolerance limits determined were found sometimes to differ very little or not at all from the corresponding 24-hour values, perhaps partly because of loss of toxicants from the test solutions. Each compound was tested with fathead minnows in soft and hard water and with bluegills and guppies in soft water at a temperature of 25° C. The four 24-hour median tolerance limits of acetonitrile,

CH_3CN, ranged from 1050 to 1850 mg/l, and the 96-hour values from 1000 to 1850 mg/l. The 24-hour and 96-hour median tolerance limits of adiponitrile, $NC(CH_2)_4CN$, for fathead minnows differed little; they were 835 and 820 mg/l in hard water, and 1350 and 1250 mg/l in soft water. However, the reported 24-hour values for bluegills and guppies were 1250 and 1200 mg/l, and the corresponding 96-hour values were 720 and 775 mg/l. Oxydipropionitrile, $(CH_3CH_2CN)_2O$, was the least toxic of the nitriles tested; its 24-hour median tolerance limits ranged from 4300 to 7350 mg/l, and the 96-hour values from 3600 to 4450 mg/l. Benzonitrile, C_6H_5CN, proved much more toxic. The 24-hour median tolerance limits for fathead minnows in hard and soft waters were reported to be 116 and 240 mg/l, respectively, and the corresponding 96-hour values 78 and 135 mg/l, respectively. The 24-hour and 96-hour values for each of the other species strangely did not differ at all; they were 78 mg/l for the bluegill and 400 mg/l for the guppy.

The principal characteristic of the soft and hard waters used by Henderson, Pickering and Lemke already have been reported in connection with the discussion of the influence of water hardness on the lethal toxicity of free cyanide. The addition only of oxydipropionitrile, of all the nitriles tested, had a considerable effect on the pH and the dissolved oxygen content of the waters, the pH of the soft water having been reduced by almost one pH unit at the highest tested concentration of this nitrile; the reduction of dissolved oxygen indicated fairly rapid oxidative degradation of the toxicant. Positive results of ordinary chemical analysis of the test solutions for cyanide were obtained only when solutions of lactonitrile and those with high concentrations of acetonitrile were tested. The measurable cyanide levels in the lactonitrile solutions were maximal about 4 hours after the beginning of the tests and declined almost to nil within 48 hours. The maxima were nearly equal to the amounts of the cyanide group introduced. The measured amounts of cyanide in the solutions of acetonitrile were reported to have been about 0.01 percent of the amounts of the cyanide group introduced, and not enough to account for the toxicity of the solutions. The concentrations actually determined in the different test solutions were not reported. My calculations show that 0.01 percent of the cyanide group present at the reported 96-hour median lethal concentration of acetonitrile for the bluegill (1850 mg/1) amounts to about 0.12 mg/1, a concentration not far below the reported 96-hour median tolerance limit of free cyanide for that species (0.15 mg/1 as CN). In the case of the fathead minnow, which was reported to be more sensitive than the bluegill to acetonitrile but less sensitive to free cyanide, the corresponding difference was much larger. It was reported that substantial hydrolysis of adiponitrile and oxydipropionitrile without release of measurable free cyanide was indicated by results of chemical tests; amounts of ammonia formed were insignificant. No evidence was observed of similar breakdown of any of the other nitriles tested.

CYANOGEN CHLORIDE

Cyanogen chloride, CNCl, which is produced upon addition of free chlorine to cyanide or thiocyanate solutions (e.g., sewage containing gas plant liquors), appears to be about as toxic to fish as free cyanide, if not more toxic. Allen, Blezard, and Wheatland (1948) have shown the lethal threshold concentration for rainbow trout at temperatures of 17-20° C to be below 0.1 mg/1 (0.04 mg/1 as CN). The toxicity tests were of short duration, apparently lasting not more than 6 hours at most, and the experimental solutions, prepared with tap water in open vessels, were not renewed during the tests. The theoretical toxicity threshold (0.08 mg/1 as CNCl) reported by the authors was derived from their data by a graphical method that is not current or approved now by fish toxicologists, but it may approximate the true lethal threshold concentration.

THIOCYANATES

The thiocyanate, CNS^-, ion itself is somewhat toxic, but not nearly as toxic as free cyanide or cyanogen chloride. Brun (1936) observed harmful effects of potassium thiocyanate, KCNS, on Gambusia holbrooki in 10-day tests only at concentrations greater than M/1000 (58 mg/l as CNS, or the equivalent of 26 mg/l of cyanide as CN). Fish exposed to M/500 and M/750 solutions survived for 2 and 6 days, respectively, in these solutions, but died within 2 and 7 days after their subsequent return to clean water. A concentration of M/75 proved lethal within 60 hours, but only a M/25 solution (2320 mg/l as CNS) killed the fish within 2 hours. The test temperatures are unknown. Schaut (1939) found that 171 mg/l as KCNS (102 mg/l as CNS) killed unidentified "minnows" within 9 or 10 days. Herbert (1962) reported that no rainbow trout were killed in a static bioassay by the highest tested concentration of sodium thiocyanate, NaCNS, which was 1800 mg/l as CNS, at 15° C and pH 7.9-8.1, but the duration of the test was not stated.

Demyanenko (1931) reported that a 1/5000 solution (200 mg/l) of ammonium thiocyanate, NH_4CNS, (153 mg/l as CNS, or 68 mg/l as CN) killed bleak, Alburnus alburnus, in about 50 hours, but a concentration half as great did not prove lethal in 144 hours. A concentration greater by about one-half (280-300 mg/l as NH_4CNS) was reported by Shelford (1917) to have been the concentration required to kill orange-spotted sunfish, Lepomis humilis, in 1 hour. But Oshima (1931) reported that a M/100 (780 mg/l) solution of NH_4CNS (580 mg/l as CNS, or 260 mg/l as CN) was tolerated by young eels, Anguilla japonica, for more than 25 hours, and a concentration 10 times as great killed the eels only after an exposure of about 3.7 hours. Thumann (1950) observed no effect on two young rainbow trout after 10 hours at a concentration of 100 mg/l as NH_4CNS, and a single rainbow trout was not visibly affected at the end of a 135-minute exposure to 2500 mg/l, but died about 10 days after its return to clean water. A young rainbow trout was killed within 150 minutes by 5000 mg/l, and a young brown trout within 135 minutes by 2500 mg/l.

Wallen, Greer, and Lasater (1957) found the 96-hour median tolerance limit of NH_4CNS for the mosquitofish, Gambusia affinis, in a turbid water at 16-23° C to be 114 mg/l (87 mg/l as CNS, or 39 mg/l as CN). However, all the fish died within 144 hours at a concentration of only 56 mg/l (43 mg/l as CNS). The 48-hour and 24-hour median tolerance limits were found to be 420 and 910 mg/l, respectively, and even 1800 mg/l did not kill most of the fish in 4.5 hours. The pH of the solutions tested by Wallen, Greer, and Lasater was reported to range from 7.4 to 7.9; the pH values of the NH_4CNS solutions tested by the other investigators are unknown. The presence of free, molecular ammonia (un-ionized base), which is highly toxic to fish, can, of course, cause or contribute to the acute toxicity of NH_4CNS solutions of sufficiently high pH. However, molecular ammonia is a rapidly fatal poison at concentrations not very much greater than the highest concentrations tolerated for long periods or indefinitely (Wuhrmann and Woker, 1948; Lloyd, 1961), whereas NH_4CNS did not kill fish very rapidly at concentrations many times greater than those that proved slowly lethal in the experiments of Wallen, Greer, and Lasater. Therefore, and in view of the reported toxicity of KCNS and other pertinent considerations, one can reasonably conclude that the toxicity of the slowly lethal test solutions of NH_4CNS probably was due mostly to the presence of the thiocyanate ion, which evidently is a slowly acting poison.

Hiatt, Naughton, and Matthews (1953a, 1953b) observed a strong irritant action of allyl and methyl isothiocyanates and of isobornyl thiocyanate or thiocyanoacetate on the marine fish Kuhlia sandvicensis. Rapid and violent reactions leading to dispersal of the schooling fish were observed at concentrations of these organic chemicals as low as 1.0 mg/l or less. A moderate (medium) reaction to as little as 0.1 mg/l of allyl isothiocyanate was reported. In the first account of their study, Hiatt, Naughton, and Matthews (1953a) reported violent reactions of the fish also to 20 mg/l of lauryl thiocyanate and 10 mg/l of phenyl isothiocyanate and of thiocyanic acid 5, 5, 5-trichloro amyl ester. Lower concentrations produced less violent reactions or were not tested. Curiously, potassium thiocyanate was reported to have produced a slight reaction at a concentration of 20 mg/l, but also, in the same publication, was included (together with ammonium and barium thiocyanates) in a list of tested chemicals that produced no observable response at that concentration. There are other discrepancies also between results reported in the two papers cited. No data have been found on the lethality to fish of any organic thiocyanates.

CYANATES

The cyanate, CNO^-, ion, which is a product of oxidation of cyanide by alkaline chlorination, a widely used method of wastewater treatment for cyanide removal, also appears to be relatively but not entirely harmless to fish. Washburn (1948) reported the tolerance limit for the creek chub, Semotilus atromaculatus, of sodium cyanate, NaCNO, to be in the neighborhood of 75 mg/l (48.5 mg/l as CNO, or 30 mg/l as CN), and Bucksteeg and Thiele (1957) reported 75 mg/l as CN to be the lower limit of concentrations harmful to fish of the potassium salt, KCNO. Enough information about the experiments performed and the results on which these values are based has not been included by the authors, however, in the cited publications. Cyanates may persist in water for a long time but are subject to hydrolysis yielding ammonium and carbonate ions.

SECTION VII

REFERENCES

Abram, F. S. H. 1964. An application of harmonics to fish toxicology. Int. J. Air Water Pollut. 8:325-338.

Achard, C., and L. Binet. 1934. Les effets de l'hyposulfite de soude sur l'intoxication par le cyanure de potassium. [The effects of sodium hyposulfite on intoxication by potassium cyanide.] C. R. Acad. Sci. 198:222-224.

Alabaster, J. S., J. H. N. Garland, I. C. Hart, and J. F. de L. G. Solbé. 1972. An approach to the problem of pollution and fisheries. Symp. Zool. Soc. London No. 29:87-114.

Alexander, W. B., B. A. Southgate, and R. Bassindale. 1935. Survey of the River Tees. Part II. The estuary--chemical and biological. Dept. of Scientific and Industrial Research (Gr. Brit.), Water Pollution Research, Technical Paper No. 5, H. M. Stationery Office, London. 171 pp. [Data on temperature effects are presented also in a summary paper: J. Mar. Biol. Assoc. U. K. 20:717-724 (1936).]

Allen, L. A., N. Blezard, and A. B. Wheatland. 1948. Formation of cyanogen chloride during chlorination of certain liquids. Toxicity of such liquids to fish. J. Hyg. 46:184-193.

American Public Health Association, *et al*. 1971. Standard methods for the examination of water and wastewater. 13th ed. Am. Pub. Health Assoc., New York. 874 pp. [See pp. 397-398.]

Anderson, P. D. 1974. An approach to the study of multiple toxicity through the derivation and use of quantal response curves. Ph.D. thesis, Oregon State University, Corvallis. 80 pp.

Armstrong, C. W. J., and K. C. Fisher. 1940. A comparison of the effects of the respiratory inhibitors azide and cyanide on the frequency of the embryonic fish heart. J. Cell. Comp. Physiol. 16:103-112.

Bandt, H-J. 1953. Acrylnitril als fischereiliches Abwassergift. [Acrylonitrile as a waste-water poison affecting fisheries.] Z. Fisch. Deren Hilfswiss., N.S. 2:457-461.

Bassindale, R., B. A. Southgate, and F. T. K. Pentelow. 1933. The effect of cyanides on the gill colour of fish. J. Mar. Biol. Assoc. U. K. 18:671-676.

Blaha, J. 1968. Zur Frage der Bestimmung und Toxozität (sic!) von freien und komplexen Cyaniden in Wässern. [On the question of determination and toxicity of free and complex cyanides in waters.] Vom Wasser 34:175-195.

Bridges, W. R. 1958. Sodium cyanide as a fish poison. U. S. Fish Wildl. Serv., Spe. Sci. Rep. Fish. No. 253. 11 pp.

Brinley, F. J. 1930. The effect of cyanide on the cardiac rhythm of embryos of Fundulus heteroclitus. Physiol. Zoöl. 3:283-290.

Brockway, D. L. 1963. Some effects of sub-lethal levels of pentachlorphenol and cyanide on the physiology and behavior of a cichlid fish, Cichlasoma bimaculatum (Linnaeus). M.S. thesis, Oregon State University, Corvallis. 56 pp.

Broderius, S. J. 1970. Determination of molecular hydrocyanic acid in water and studies of the chemistry and toxicity to fish of the nickelocyanide complex. M.S. thesis, Oregon State University, Corvallis. 93 pp.

Broderius, S. J. 1973. Determination of molecular hydrocyanic acid in water and studies of the chemistry and toxicity to fish of metal-cyanide complexes. Ph.D. thesis, Oregon State University, Corvallis. 287 pp.

Brown, V. M., D. G. Shurben, and D. Shaw. 1970. Studies of water quality and the absence of fish from some polluted English rivers. Water Res. 4:363-382.

Brun, P. 1936. Sur la toxicité relative des ions thiocyanique. [On the relative toxicity of thiocyanate ions.] C. R. Soc. Biol. 121:543-546.

Bucksteeg, W. 1961. Teste zur Beurteilung von Abwässern. [Tests for the examination of waste waters.] Staedtehygiene 12(9):180-184.

Bucksteeg, W., and H. Thiele. 1957. Möglichkeiten zur experimentellen Ermittlung der Einhohnergleichwerte für industrielle Abwässer. [Possibilities of experimental assessment of population equivalents for industrial wastes.] Gas Wasserfach 98:909-910 (Wasser - Abwasser pp. 465-466).

Burdick, G. E. 1957. A graphical method for deriving threshold values of toxicity and the equation of the toxicity curve. N. Y. Fish Game J. 4:102-108.

Burdick, G. E., H. J. Dean, and E. J. Harris. 1958. Toxicity of cyanide to brown trout and smallmouth bass. N. Y. Fish Game J. 5:133-163.

Burdick, G. E., and M. Lipschuetz. 1950. Toxicity of ferro- and ferricyanide solutions to fish, and determination of the cause of mortality. Trans. Am. Fish. Soc. 78[for 1948]:192-202.

Cairns, J., Jr., and A. Scheier. 1958. The effect of periodic low oxygen upon toxicity of various chemicals to aquatic organisms. Proc. 12th Ind. Waste Conf., Eng. Bull. Purdue Univ., Eng. Ext. Ser. No. 94 [Eng. Bull., 42(3)]:165-176.

Cairns, J., Jr., and A. Scheier. 1959. The relationship of bluegill sunfish body size to tolerance for some common chemicals. Proc. 13th Industr. Waste Conf., Eng. Bull. Purdue Univ., Eng. Ext. Ser. No. 96[Eng. Bull. 43(3)]:243-252.

Cairns, J., Jr., and A. Scheier. 1963. Environmental effects upon cyanide toxicity to fish. Not. Nat. Acad. Nat. Sci. Philadelphia, No. 361. 11 pp.

Cairns, J., Jr., and A. Scheier. 1968. A comparison of the toxicity of some common industrial waste components tested individually and combined. Prog. Fish-Cult. 30:3-8.

Cairns, J., Jr., A. Scheier, and J. J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish *Lepomis machrochirus* Raf. Not. Nat. Acad. Nat. Sci. Philadelphia, No. 381. 9 pp.

Carter, L. 1962. Bioassay of trade wastes. Nature (London) 196:1304.

Chan, K. K-S. 1971. Some effects of chronic cyanide poisoning on osmoregulation of rainbow trout. M.S. thesis, Sir George Williams University, Montreal, Canada. [Abstract only seen.]

Chen, C. W., and R. E. Selleck. 1969. A kinetic model of fish toxicity threshold. J. Water Pollut. Control Fed. 41(8, Pt.2):R294-R308.

Christensen, J. J., R. M. Izatt, J. D. Hale, R. T. Pack, and G. D. Watt. 1963. Thermodynamics of metal cyanide coördination. II. ΔG^0, ΔH^0, and ΔS^0 values for tetracyanoniccolate(II) ion formation in aqueous solution at 25°. Inorg. Chem. 2:337-339.

Claeys, R. R., and H. Freund. 1968. Gas chromatographic separation of HCN on Porapak Q: Analysis of trace aqueous solutions. Environ. Sci. Technol. 2:458-460.

Costa, H. H. 1965. Responses of freshwater animals to sodium cyanide solutions. 1. Fish. Ceylon J. Sci. Biol. Sci. 5(2):41-87.

Costa, H. H. 1966. The effect of exercise on the survival of Phoxinus phoxinus L. in sodium cyanide solutions. Hydrobiologia 28(2):241-251.

Daugherty, F. M., and T. J. Garrett. 1951. Toxicity levels of hydrocyanic acid and some industrial by-products. Tex. J. Sci. 3:391-396.

Demyanenko, V. N. 1931. Poisoning of fish by waste waters from chemical factories and the "fish test" [in Russian]. Gig. Epidemiol. 10(6/7):13-20.

Dorier, A. 1952. Toxicité pour les poissons de cyanure de sodium industriel. [Toxicity to fish of industrial sodium cyanide.] Trav. Lab. Hydrobiol. Pisc. Grenoble 43-44[for 1951-52]:103-106.

Doudoroff, P. 1956. Some experiments on the toxicity of complex cyanides to fish. Sewage Ind. Wastes 28:1020-1040.

Doudoroff, P. 1965. Formal Discussion (of paper by S. Ishio, "Behavior of fish exposed to toxic substances"). Pages 33-35 in O. Jaag, ed. Advances in water pollution research (Proc. Int. Conf. Int. Assoc. Water Pollut. Res.), 1964, Vol. 1. Permagon Press, Inc., New York.

Doudoroff, P., and M. Katz. 1950. Critical review of literature on the toxicity of industrial wastes and their components to fish. I. Alkalies, acids, and inorganic gases. Sewage Ind. Wastes 22:1432-1458.

Doudoroff, P., G. Leduc, and C. R. Schneider. 1966. Acute toxicity to fish of solutions containing complex metal cyanides, in relation to concentrations of molecular hydrocyanic acid. Trans. Am. Fish. Soc. 95:6-22.

Downing, K. M. 1954. The influence of dissolved oxygen on the toxicity of potassium cyanide to rainbow trout. J. Exp. Biol. 31:161-164.

Ellis, M. M. 1937. Detection and measurement of stream pollution. U. S. Bur. Fish. Bull. No. 22. Bull. Bur. Fish. 48:365-437.

Fisher, K. C., and R. Ohnell. 1940. The steady state frequency of the embryonic fish heart at different concentrations of cyanide. J. Cell. Comp. Physiol. 16:1-13.

Freund, H., and C. R. Schneider. 1959. Determination of the cumulative dissociation constant of tetracyanonickelate(II) ion. J. Am. Chem. Soc. 81:4780-4783.

Gillar, J. 1962. The effect of cyanide on some aquatic animals [in Czech, with Russian and German summaries]. Sb. Vys. Sk. Chem.-Technol. Praze, Technol. Vody 6(Pt. 1):435-457.

Grau, P., and J. Hrubec. 1965. Reasons and course of the disastrous pollution of the Jihlava River with cyanide [in Czech, with English and Russian summaries separately provided]. Vodni Hospod. 15(1):19-20.

Great Britain, Department of Scientific and Industrial Research. 1953. Water pollution research 1952. H. M. Stationery Office, London. [See pp. 42-45.]

Great Britain, Department of Scientific and Industrial Research. 1956. Water pollution research 1955. H. M. Stationery Office, London. [See pp. 37-38.]

Great Britain, Department of the Environment. 1972. Water pollution research 1971. H. M. Stationery Office, London. [See pp. 38-40.]

Great Britain, Department of the Environment. 1973. Water pollution research 1972. H. M. Stationery Office, London. [See pp. 39-41.]

Great Britain, Ministry of Technology. 1967. Water pollution research 1966. H. M. Stationery Office, London. [See pp. 152-154.]

Great Britain, Ministry of Technology. 1968. Water pollution research 1967. H. M. Stationery Office, London. [See pp. 60-65.]

Great Britain, Ministry of Technology. 1969. Water pollution research 1968. H. M. Stationery Office, London. [See pp. 61 and 65.]

Great Britain, Ministry of Technology. 1970. Water pollution research 1969. H. M. Stationery Office, London. [See pp. 63-64.]

Henderson, C., Q. H. Pickering, and A. E. Lemke. 1961. The effects of some organic cyanides (nitriles) on fish. Proc. 15th Ind. Waste

Conf., Eng. Bull. Purdue Univ., Eng. Ext. Ser. No. 106 [Eng. Bull. 45(2)]:120-130.

Herbert, D. W. M. 1962. The toxicity to rainbow trout of spent still liquors from the distillation of coal. Ann. Appl. Biol. 50:755-777.

Herbert, D. W. M., and K. M. Downing. 1955. A further study of the toxicity of potassium cyanide to rainbow trout (Salmo gairdneri Richardson). Ann. Appl. Biol. 43:237-242.

Herbert, D. W. M., K. M. Downing, and J. C. Merkens. 1955. Studies on the survival of fish in poisonous solutions. Int. Ver. Theor. Angew. Limnol., Verh. 12:789-794.

Hiatt, R. W., J. J. Naughton, and D. C. Matthews. 1953a. Effects of chemicals on schooling fish, Kuhlia sandvicensis. Biol. Bull. (Woods Hole, Mass.) 104:28-44.

Hiatt, R. W., J. J. Naughton, and D. C. Matthews. 1953b. Relation of chemical structure to irritant responses in marine fish. Nature (London) 172:904-905.

Hubault, E. 1955a. Étude sur la progressivité de la pollution de la Meurthe en fonction du débit et sur les seuils de nocivité de divers composés chimiques vis-à-vis du poisson. [Study of the progress of pollution of the Meurthe in relation to the flow and of the toxicity thresholds of various chemical compounds for fish.] Eau 42(11):271-276.

Hubault, E. 1955b. Les seuils de nocivité de divers composés chimiques vis-à-vis du poisson. [The toxicity thresholds of various chemical compounds for fish.] C. R. 27th Congr. Int. Chim. Ind. (Brussels) 1954 1; Ind. Chim. Belge 20 (Spec. No.):352-356.

Ishida, J. 1947. Mechanism of opercular movement by the embryo of killifish [in Japanese]. Dobutsugaku Zasshi 57(1/2):14-15. Biol. Abs. (1950) 24:635, Abs. No. 6446. [Indirectly cited.]

Ishio, S. 1965. Behavior of fish exposed to toxic substances. Pages 19-33 in O. Jaag, ed. Advances in water pollution research (Proc. Int. Conf. Int. Assoc. Water Pollut. Res.), 1964, Vol. 1. Permagon Press, Inc., New York.

Jackson, S., and V. M. Brown. 1970. Effect of toxic wastes on treatment processes and watercourses. Water Pollut. Control 69:292-303.

Jones, J. R. E. 1947. The oxygen consumption of Gasterosteus aculeatus L. in toxic solutions. J. Exp. Biol. 23:298-311.

Kariya, T., R. Akiba, S. Suzuki, and T. Tsuda. 1967. Studies on the post-mortem identification of the pollutant in the fish killed by water pollution. IV. Detection of cyanide in fish [in Japanese, with English abstract, table headings, and figure legends]. Bull. Jpn. Soc. Sci. Fish. (Tokyo) 33(4):311-314.

Karsten, A. 1934. Investigation of the effect of cyanide on Black Hills trout. Black Hills Eng. 22:145-174.

Leclerc, E., and F. Devlaminck. 1950. Etude toxicologique de quelques substances généralement présents dans les effluents d'usines à gaz. [Toxicological studies of some substances usually present in effluents from gas works.] Cent. Belge Etude Doc. Eaux, Bull. Mens. No. 8 [1950/II]:486-493.

Leduc, G. 1966. Some physiological and biochemical responses of fish to chronic poisoning by cyanide. Ph.D. thesis, Oregon State University, Corvallis. 146 pp.

Leschber, R. 1969. Die Beurteilung der Toxizität cyanidhaltiger Abwässer. Zur Frage der Beziehung zwischen der Toxizität und der analytischen Bestimmung von Cyaniden durch geeignete Verfahren. [The estimation of the toxicity of cyanide-containing wastewaters. On the question of the relation between the toxicity and the analytical determination of cyanides by appropriate methods.] Galvanotechnik 60:368-374.

Lewis, W. M., and R. M. Tarrant, Jr. 1960. Sodium cyanide in fish management and culture. Prog. Fish-Cult. 22:177-180.

Lipschuetz, M., and A. L. Cooper. 1955. Comparative toxicities of potassium cyanide and potassium cuprocyanide to the western black-nosed dace (Rhinichthys atratulus meleagris). N. Y. Fish Game J. 2:194-204.

Llyod, R. 1961. The toxicity of ammonia to rainbow trout. Water Waste Treatment J. 8:278-279.

Lloyd, R., and D. H. M. Jordan. 1963. Predicted and observed toxicities of several sewage effluents to rainbow trout. Inst. Sewage Purif., J. Proc. 1963 (Pt.2):167-173.

Lloyd, R., and D. H. M. Jordan. 1964. Predicted and observed toxicities of several sewage effluents to rainbow trout: A further study. Inst. Sewage Purif., J. Proc. 1964 (Pt. 2):183-186.

Malacea, I. 1966. Contributions to knowledge of the toxic effects of cyanides, ammonia, mercury, and arsenic on some species of fish and on *Daphnia* [in Roumanian, with English, German, and Russian summaries]. Stud. Prot. Epurarea Apelor *7*(2):751-792.

Malacea, I. 1968. Untersuchungen über die Gewöhnung der Fische an hohe Konzentrationen toxischer Substanzen. [Studies on the acclimatization of fishes to high concentrations of toxic substances.] Arch. Hydrobiol. *65*:74-75.

Michigan Department of Conservation, Institute for Fisheries Research. 1933. The toxicity to fish life of cyanide solutions, and of the products derived from certain chemical treatments of cyanide solutions. Rep. No. 207, Inst. Fish. Res., University of Michigan, Ann Arbor. Typescript or mimeo. (photostatic copy seen). 14 pp.

Milne, D. 1950. Disposal of cyanides by complexation. Sewage Ind. Wastes *22*:1192-1199.

Montgomery, H. A. C., D. K. Gardiner, and J. G. G. Gregory. 1969. Determination of free hydrogen cyanide in river water by a solvent-extraction method. Analyst (London) *94*:284-291.

Moore, S. L., and S. R. Kin. 1968. Cyanide pollution and emergency duty, train wreck, Dunreith, Indiana. Proc. 23rd Industr. Waste Conf., Eng. Bull. Purdue Univ., Eng. Ext. Ser. No. 132 (Pt. 1) [Eng. Bull. *53*(2)]:583-600.

Myers, C. S., and T. Iezzi. 1950. A report of experiments on the effect of certain toxicants on fish life with especial reference to the effect of photodecomposition. Mimeo. Rep., Ind. Wastes Div., Bur. Sanit. Eng., Pennsylvania Department of Health, Harrisburg, Pa. 10 pp.

Negilski, D. S. 1973. Individual and combined effects of cyanide, pentachlorphenol and zinc on juvenile chinook salmon and invertebrates in model stream communities. M.S. thesis, Oregon State University, Corvallis. 80 pp.

Nehring, D. 1964. Die Schadwirkung von Kupfersulfat, Zinksulfat, Kaliumzyanid, Ammoniak und Phenol gegenüber Karpfen (*Cyprinnus carpio*) vom Wasser her und nach peroraler Applikation. [The toxicity to carp (*Cyprinus carpio*) of copper sulfate, zinc sulfate, potassium cyanide, ammonia, and phenol from water and after oral administration.] Z. Fisch. Deren Hilfswiss., N.S. *12*:717-724.

Neil, J. H. 1957. Some effects of potassium cyanide on speckled trout (*Salvelinus fontinalis*). Pages 74-96 *in* Papers presented at fourth

Ontario industrial waste conference. Water and Pollution Advisory Committee, Ontario Water Resources Commission, Toronto, Canada.

Nelson, K. H., and I. Lysyj. 1971. Analysis of water for molecular hydrogen cyanide. J. Water Pollut. Control Fed. 43:799-805.

Oshima, S. 1931. On the toxic action of dissolved salts and electrolytes upon young eels (Anguilla japonica) [in Japanese]. J. Imp. Fish. Exp. Sta. (Tokyo) No. 2:139-193.

Patrick, R., J. Cairns, Jr., and A. Scheier. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. Prog. Fish-Cult. 30:137-140.

Phillips, F. S. 1940. Oxygen consumption and its inhibition in the development of Fundulus and various pelagic fish eggs. Biol. Bull. (Woods Hole, Mass.) 78:256-274.

Powers, E. B. 1917. The goldfish (Carassius carassius) as a test animal in the study of toxicity. Ill. Biol. Monogr. 4(4):123-193 [or pp. 3-73 of No. 4 only].

Renn, C. E. 1955. Biological properties and behaviors of cyanogenic wastes. Sewage Ind. Wastes 27:297-308.

Schaut, G. G. 1939. Fish catastrophies during droughts. J. Am. Water Works Assoc. 31:771-821.

Schneider, C. R., and H. Freund. 1962. Determination of low level hydrocyanic acid in solution using gas-liquid chromatography. Anal. Chem. 34:69-74.

Seeberger, X. 1921. Toxische Wirkung von Brennereiruckstanden auf Fische. [Toxic action of distillery wastes on fish.] Verh. Schweiz. Naturforsch. Ges. 102(Pt. 2):193-195.

Seth, A. K., S. K. Shrivastava, M. G. George, and J. K. Bewtra. 1967. Monitoring of certain toxic constituents in water supplies. Environ. Health 9:34-38.

Shelford, V. E. 1917. An experimental study of the effects of gas wastes upon fishes, with especial reference to stream pollution. Bull. Ill. State Lab. Nat. Hist. 11:381-412 + figure and charts with captions (12 pp).

Silaichuk, E. V. 1969. Effect of waste waters containing cyanides and hexavalent chromium on the survival of certain aquatic organisms [in Russian]. Gig. Sanit. 34(12):78-79.

Southgate, B. A. 1932. The toxicity of mixtures of poisons. Q. J. Pharm. Pharmacol. 5:639-648.

Southgate, B. A., F. T. K. Pentelow, and R. Bassindale. 1932. An investigation into the causes of death of salmon and sea trout smolts in the estuary of the River Tees. Biochem. J. 26:273-284.

Southgate, B. A., F. T. K. Pentelow, and R. Bassindale. 1933. The toxicity to trout of potassium cyanide and p-cresol in water containing different concentrations of dissolved oxygen. Biochem. J. 27:983-985.

Sprague, J. B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4:3-32.

Stumm, W., H. Woker, and H. U. Fischer. 1954. Die Entgiftung von zyanidhaltigen Abwässern durch Oxydation mit Hypochlorit. [Detoxification of waste waters containing cyanide by oxidation with hypochlorite.] Schweiz. Z. Hydrol. 16:1-21.

Summerfelt, R. C., and W. M. Lewis. 1967. Repulsion of green sunfish by certain chemicals. J. Water Pollut. Control Fed. 39:2030-2038.

Sumner, F. B., and P. Doudoroff. 1938. Some experiments on temperature acclimatization and respiratory metabolism in fishes. Biol. Bull. (Woods Hole, Mass.) 74:403-429.

Sumner, F. B., and M. C. Sargent. 1940. Some observations on the physiology of warm spring fishes. Ecology 21:45-54.

Sumner, F. B., and N. A. Wells. 1935. Some relations between respiratory metabolism in fishes and susceptibility to certain anesthetics and lethal agents. Biol. Bull. (Woods Hole, Mass.) 69:368-378.

Symons, G. E., and R. W. Simpson. 1939. Report on fish destruction in the Niagara River. Trans. Am. Fish. Soc. 68[for 1938]:246-255.

Thumann, M.-E. 1950. Über die Wirkung von Ammoniumsaltzlösungen auf Regenbogen- und Bachforellen und einige Fischnährtiere. [On the action of ammonium salt solutions on rainbow and brown trout and on some fish-food organisms.] Abh. Fisch. Deren Hilfswiss. 1950, Pt. 2:327-348.

Turnbull, H., J. G. DeMann, and R. F. Weston. 1954. Toxicity of various refinery materials to fresh-water fish. Ind. Eng. Chem. 46:324-333.

Wallen, I. E., W. C. Greer, and R. Lasater. 1957. Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage Ind. Wastes 29:695-711.

Warren, C. E. (with P. Doudoroff). 1971. Biology and water pollution control. W. B. Saunders Co., Philadelphia. 434 pp.

Washburn, G. N. 1948. The toxicity to warm-water fishes of certain cyanide plating and carburizing salts before and after treatment by the alkali-chlorination method. Sewage Works J. 20:1074-1083.

Weiss, B., H. A. Abramson, and M. O. Baron. 1958. Lysergic acid diethylamide (LSD-25). XXV. Effect of potassium cyanide and other oxidase and respiratory inhibitors on the Siamese fighting fish. Arch. Neurol. Psychiatry 80:345-350.

Wells, M. M. 1916. Starvation and the resistance of fishes to lack of oxygen and to KCN. Biol. Bull. (Woods Hole, Mass.) 31:441-452.

Woker, H., and K. Wuhrmann. 1950. Beiträge zur Toxicologie der Fische. VI. Die Empfindlichkeit verschiedener Fischarten gegenüber Ammoniak, Blausäure und Phenol. [Contributions to fish toxicology. VI. The sensitivity of different species of fish to ammonia, hydrocyanic acid, and phenol.] Rev. Suisse Zool. 57(3):548-553.

Worley, F. P., and V. R. Browne. 1917. The hydrolysis of sodium cyanide. J. Chem. Soc. (Trans.) 111:1057-1062.

Wuhrmann, K. 1952. Sur quelques principes de la toxicologie du poisson. (Concerning some principles of the toxicology of fishes.) Cent. Belge Etude Doc. Eaux, Bull. Mens. No. 15 [1952/I]:49-60.

Wuhrmann, K., and H. Woker. 1948. Beiträge zur Toxicologie der Fische. II. Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. [Contributions to fish toxicology. II. Experimental investigations of ammonia and hydrocyanic acid poisoning.] Schweiz. Z. Hydrol. 11:210-244.

Wuhrmann, K., and H. Woker. 1953. Beiträge zur toxicologie der Fische. VIII. Über die Giftwirkungen von Ammoniak- und Zyanidlösungen mit verschiedener Sauerstoffspannung und Temperatur auf Fische. [Contributions to fish toxicology. VIII. Concerning the toxicity to fishes of ammonia and cyanide solutions with varying oxygen tension and temperature.] Schweiz. Z. Hydrol. 15:235-260.

Wuhrmann, K., and H. Woker. 1955. Influence of temperature and oxygen tension on the toxicity of poisons to fish. Int. Ver. Theor. Angew. Limnol., Verh. 12:795-801.